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CLINICAL AND EXPERIMENTAL

THE PATHOLOGIST OF 1940*

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I DO not know why our worthy secretary suggested this title unless he was anxious to learn, in detail, what I failed to say in two recent papers. Last year before this society I spoke upon "The Pathologist of the Twentieth Century" and last January I spoke before the Congress of Internal Medicine upon "The Making of a Doctor." In both of those addresses ran a progressive theme which I hope I have not completed; there is much yet to be said relative to the present stage of evolution of our profession and the possibilities for the future. We are certainly in a transitional stage.

Some human minds, interested in the problem of medical progress, have the faculty of attempting to present plans broadly in order to stimulate others to work out details. This type of mind may work in two ways: it may make observations, correlations, and generalizations and present hypotheses or plans which are heartily approved; or it may stimulate others to prove them incorrect. In either case a service is rendered. It was with an idea of such service that both papers were presented. In them I attempted to study the historical evolution of the profession of medicine and see its relationship to the individual we have correctly or incorrectly called the pathologist. I also attempted to analyze the evolution, the functions of, and the service actually rendered by the various so-called specialists who deal with disease. To this analysis was added a study of the economic factors as well as the actual efficiency of our practice of medicine.

We have never been business men and we have never made any claims to being a profession which deals with exact sciences. Captains of industry, mathematicians, physicists and engineers put us to shame; and we cover our

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superficialities and inexactitudes by grouping ourselves with the learned professions of theology and law. I am not familiar with theology although I know that metaphysical thinking is its chief attribute; I am, however, sufficiently acquainted with law to know that leading authorities have difficulty in their attempts to define jurisprudence as a science.

If you study our own profession carefully and with an unprejudiced mind you will see that the only important subject, upon which our practice is based, which has any semblance of accuracy, is normal anatomy. It has taken many centuries to develop it. Although systematic study of signs and symptoms is also very ancient, the real significance of signs and symptoms has become apparent only in very recent years. Their actual significance was not appreciated until the beginning of the last half of the last century and then not until we began to systematically study morbid anatomy in relation to them. It was the individual who realized the necessity of putting the two together whom we called the pathologist. He was a physician who, to his knowledge of symptoms and signs, added first-hand information about structure and anatomical location of disease. It was such an individual who knew the most about disease. He became the authority upon the subject pathology—the science of disease, its nature, cause and course. His field was large; much careful and detailed exploration was needed; it required his whole time; actual practice, on his part, was of necessity neglected. He founded a new and basic branch of medicine which was unfortunately called a specialty. He became rapidly a thing apart from his brother physicians; he lost much; our science gained much; and those who still clung to signs and symptoms alone remained largely in the same class with the ancient physicians. Some of our practitioners and consultants today are still in that class; others by reading have learned, in a second-hand fashion, to correlate their symptoms and signs with the reported structural findings.

The dividing of our investigative ways was necessary like the early diverse paths of exploration of America. Some approached the west by a northern route, some the southern route, and others by a middle route; but today we go in all directions and meet at any point. And so it has been in medicine, the trails, especially in our generation, have been parallel, sometimes divergent and sometimes convergent; they have frequently crossed each other. This last condition I believe we are in now; our trails of scientific progress are crossing each other; we are unnecessarily retracing each other's steps. It is time, it seems to me, to form trunk lines and eliminate many valueless cross lines. This we see in our attempts at group medicine and hospital standardization. This is all very good but what have we done and what are we doing? We are developing physical examiners of patients, chest experts, rectal experts, nose and throat experts, gynecologic experts, surgical operators, radio-therapists, skin specialists, kidney specialists, bladder specialists, head specialists—in fact specialists for every anatomical region and organ of the body. To these we have added laboratory specialists for blood, urine, feces, and other body fluids and excretions. Even the business and social service sides of our professional work have been specialized. We are all specialists but where are the great correlators and generalizers? What is the basis of correlation of facts about

disease? These are the questions, the answers to which will point the way we are going as a profession.

In my opinion there are only two answers: we, as a lot of specialists, are going to be managed by executives who are business men primarily; or we will develop individuals who, by correct and long training, know how to put all of the facts, found by experts, together and read the diagnosis, cause, prevention, palliation, and cure of disease with its social, economic and humanitarian significance. This individual will, by original definition, be the pathologist. He will not be a morbid anatomist alone, serologist alone, symptomologist alone, roentgenologist alone, immunologist alone, surgeon alone, therapist alone, or any other kind of narrow specialist alone. He will be one who has had the broadest experience with all. He cannot be a youth, although his mind may be youthful. He cannot be made in a few years; he must have spent a long apprenticeship: he must have the needs of the whole patient at heart.

Let us not mislead and narrow ourselves in our great haste to succeed in fame or finance. Let us face the facts and realize that it takes time to master the art and science of dealing with disease. Let us not mistake business acumen for the art of practice or the show of delicate instruments and tiled laboratories for science. The question we should ask ourselves constantly is, what do we actually do in the diagnosis, palliation, and cure of disease, and do we do it in the most efficient and economical manner for the patient? Let us classify and card index our knowledge and activities into the following groups:

1. Those of actual immediate value.
2. Those of possible future value.
3. Those of teaching value.
4. Those of no value.

A business man or an engineer would, with his type of mind, demand that we do this before he would consider us efficient.

The real pathologist of 1940 will have this type of mind and will know the most about disease as it affects the human being as a whole.

It is my hope that this society which bears the name of pathologists will have this as its ideal and not merely the ideal of developing well organized expert laboratory technicians. If you do your job well and deserve the name of pathologists you will be of value as consultants in every specialty and to every physician and patient in your community. There will always be those who will disparage your work and methods to save their own necks and pocketbooks, but some day, perhaps in 1940, laymen will be seeking your advice and not be running away from our specialists to chiropraxy, osteopathy, Christian science, corner drug stores, and Abramism, as they are doing today. What we are trying to do with legislation we shall have done by public and professional education.

And now just to show you that what I have said is not the speech of an idealist without practical experience, may I state a few facts and ask a few questions for your consideration.

In the hands of the best of clinicians we find in a series of 60,645 patients there were 225,785 laboratory reports (5,650 metabolic basal rate determina-

tions, 11,946 gastric analyses, 192,739 urinalyses and other laboratory procedures, 2,021 electrocardiographic records, 13,964 fresh tissue examinations during operations and for purely diagnostic purposes and 465 necropsies). These required time, material, space and human energy. What percentage of these were of actual positive or negative value? How many of them would have been necessary in the hands of clinicians who were also well trained pathologists or pathologists who were keen observers of signs and symptoms?

For sixteen years I have studied 1000 consecutive surgical cases for each year and attempted to determine what percentage of them failed to have absolutely positive and unqualified clinical preoperative diagnoses. In the 16,000 cases the percentage was 24.66. This percentage figure has increased from 25 per cent in the first year to 36.7 per cent in the last year despite our increase in laboratory diagnostic methods and facilities. There are several possible explanations: (1) The character of the cases may have changed. (2) The clinicians may not take enough time to study their patients closely, depending upon laboratory methods or exploration operations for diagnosis: (3) We may be seeing an increasing number of early lesions which do not present differential signs and symptoms. The first does not explain the difference in percentage because actual study does not reveal a great difference in the character of patients. The second reason is at least partially correct because medical students and recent graduates are not being taught clinical observation; they are spending most of their time upon the laboratory study of disease. The third presents an actual reason. Those of us who have seen a large series of pathologic conditions over a long period of years know that the conditions are actually in earlier stages when we see them; patients and general physicians are becoming better educated to the necessity of early diagnosis and early treatment, and surgical exploration is becoming a diagnostic method.

The increasing frequency of doubtful clinical diagnoses and exploratory operations is not a detrimental reflection upon our ability as diagnosticians: it is, on the contrary, a sign of our diagnostic honesty and desire to put medicine upon an exact basis. We are beginning to know when we do not know. We refuse to guess and deceive ourselves.

In the last one thousand cases of this series there was a 9.9 per cent actual error in anatomical location and type of lesion with different prognostic and therapeutic values. This is a significant figure and might have been tragic if the surgeons had not been capable of dealing with all types of conditions and had not had detailed microscopic diagnosis at hand. In no instance in this series was the clinical diagnostic failure tragic. The figure merely shows what can and cannot be done with gross clinical symptoms and signs alone. It shows how frequently mistakes in diagnosis are being made when not controlled by exploration and immediate pathologic* examinations.

In 168 malignant conditions only 70.2 per cent were diagnosed positively malignant by the clinicians. Two and five-tenths per cent were diagnosed benign and in 27.3 per cent malignancy was suspected as a possibility.

With all due respect and credit to clinicians who have dealt largely with

*The term *pathologic* is used here to mean microscopic.

signs and symptoms, I feel that these figures might have been different in the hands of those who know morbid anatomical conditions and possibilities.

I feel quite certain that no physician knows as much about the abdomen as an experienced abdominal surgeon. I also feel that no surgeon knows as much about the tissues he deals with as the one who dissects them and studies them microscopically. Our physicians of today, as also of yesterday, do not know gastric ulcer, duodenal ulcer, hepatitis, cholecystitis, tuberculosis of the kidneys, pancreatitis, appendicitis, ovarian conditions, uterine conditions if they have not constantly correlated the clinical pictures with the actual appearances at exploration. It is one thing to read a surgical and pathological report and another to actually see the conditions. No physician can be a great pathologist until he has seen disease in the living patient. He cannot learn the most about disease unless he actually checks his clinical judgment.

I mention these things merely to stimulate you to correlate your laboratory experiences with the condition of your patients. Cling closely to your surgical friends and incidentally have them cling to you that their judgment may also improve.

And now I imagine I hear you saying what I have actually heard: "You are doing your laboratory work under ideal and very different circumstances; you do not know our problems out in practice."

Perhaps this is true, but here are your problems. A doctor sends you a specimen (blood, urine, feces, or tissue) or perhaps the patient for examination of his secretions, excretions, body fluids or his tissues and you make the examination and send a report. In most instances you are completely through with the patient; you make a report or diagnosis of that which you have examined. As a rule you do not make the diagnosis of the patient's condition. Your knowledge of the disease is incomplete: you are not a pathologist, you are an expert technician. Not until you render clinical judgment in the case do you step into the field of the practice of medicine. Perhaps your personal interest is merely the technical laboratory side of disease. If so, you are just like the brick-layer who lays the bricks after the architect has told him where to put them. If you want to be a brick-layer, well and good; if you want to be an architect in medicine then you must study not only brick-laying, but everything else about construction, strength of materials, cost, and time.

Desultory as my remarks have been I hope they convey the following points: the real pathologist—the one who deserves the name—is the one who knows the most about disease. No clinician can become an expert until he knows very well from actual experience the relation of morbid anatomy and pathologic processes to signs and symptoms, and no so-called pathologist can be an expert pathologist until he knows the clinical values of his knowledge of morbid anatomy and processes.

The pathologist of 1940 will be the greatest correlator of all the facts about disease from the standpoint of the whole patient and not a narrow specialist upon some organ or technical laboratory procedure.

Such a well-trained pathologist with a systematic investigative mind can direct any laboratory procedure, evaluate the procedure and correlate the findings with his physical and psychical examinations. Such pathologists will, if

I see correctly, be the leading medical consultants in the year 1940. They will know the fundamental differences between investigation, teaching and application. They will not mix them quite as indiscriminately as they are mixed today in our efforts to carry on our professional activities.

This Society, with the high ideals which have become so evident in its members who are young and progressive, will tend to make many pathologists such as I have described for 1940.

THE CLINICAL VALUE OF VITAL STAINING*

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VITAL staining is not a new contribution to medical progress. Ehrlich, in 1886, was probably the first to demonstrate its usefulness, and to advocate its adoption. For many years it received little attention until revived by Wilson who applied it in the diagnosis of tissue fresh from the operating room. MacCarty has since applied it in the study of cancer cells. The technic of its application is simple, and with the improved and rapid method of preparing the various stains it offers a means whereby living tissue can be studied without subjecting it to changes produced by fixing, baking and embedding.

Just what occurs during the process of staining living tissues is still a matter of speculation. It is generally assumed that the membranes of living cells are a mixture of lipoids and protein colloids, and that they both play a part in the penetration. While it is true that certain lipoid soluble substances penetrate the cells more readily than those which are only water soluble, many substances penetrate independently of the lipoid solubility. It has been suggested that permeability is selective, apparently independent of molecular size; it is extremely sensitive to slight disturbances. Undoubtedly, physical and chemical factors are involved in the phenomenon, and most of the chemical reactions which occur are due to ionization. Proteins, phospholipins and carbohydrates are involved in these reactions. Living cells are probably semipermeable, the phenomenon being dependent on a selective reaction or solubility. After the dye or stain penetrates, reactions probably occur between ions. The reaction of the living cells is usually of such a character that the proteins and phospholipins (mainly the former) are present in part as negatively charged ions, and thus in a favorable form for combining with neutral or basic dyes. The cell contents would have to become distinctly acid before proteins would be present as positively charged particles, and thus able to combine with acid dyes. Death favors the development of acidity, but even then we would expect the simple proteins, such as the albumins and globulins, to react as bases. With the conjugated neucleo-

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proteins, glycoproteins or phosphoproteins, a very much higher hydrogen-ion concentration (lower pH) would be required to cause these complex proteins to ionize as positively charged particles.

It seems, therefore, that the selective permeability of living cells is the important factor, the loss of which enables dead cells to take stains more readily. That acid dyes are generally much less toxic than basic is well known. The vital staining qualities of the acid and basic dyes are not parallel. Harvey has shown that living tissue resists the entrance of all acids (except salicylic acid) while dead tissues are freely permeable to them. Selective permeability persists only during life. It is lost when cells die, so that dead cells merely imbibe solutions like gelatin or fibrin. In vital staining we are, therefore, chiefly concerned with the neutral and basic dyes.

It is well known that the reaction of living matter can be determined by the use of indicators or dyes, because of the color reactions produced when they pass from an alkali to an acid reaction. As stated by Mathews, it is

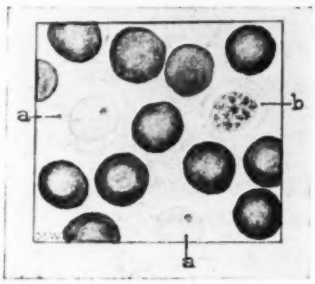

Fig. 1.—Lymphoblasts in lymphatic leukemia. *(a)* Lymphoblasts; *(b)* lymphocyte.

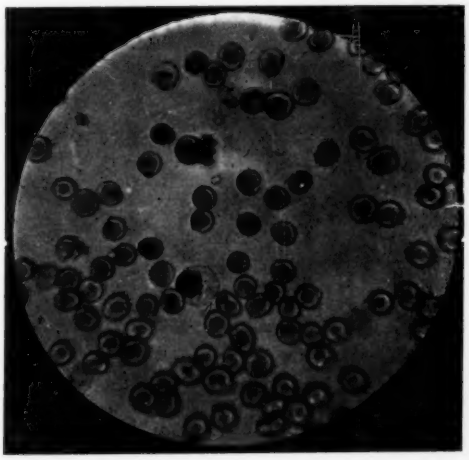

Fig. 2.—Polymorphonuclear leukocyte with clear area of cytoplasm.

thus quite possible to determine the viability of cells, as various experiments have shown that protoplasm is generally faintly alkalin in reaction and that it becomes acid after death, or when subjected to a medium with too little oxygen. Neutral red penetrates nearly all cells with ease, and stains the granules in the cells and sometimes the other structures. It always takes the color it has in an alkaline reaction, and furthermore, it is found that when protoplasm is made acid, it no longer stains with neutral red. Acid fuchsin will not stain protoplasm so long as it is alkalin, but will do so as soon as it is made acid. Vital stains must, therefore, be nontoxic and must be capable of combining with some element in the cells in order to accumulate in protoplasm.

For convenience the vital stains are divided into two groups: the toxic, of which methylene blue and polychrome-methylene blue are the most important, and the nontoxic, of which brilliant green and neutral red are com-

monly employed. It is the polychrome-methylene blue of the toxic group of vital stains which is of prime importance, for this stain may serve two purposes: a study of fresh tissue in its normal condition, and a study of the fixed preparation. It is thus possible to study the fresh tissue and a smear of cells with the same stain.

During a study of the process of tissue staining I became interested in the study of fresh blood, and directed my attention to the possibility of recognizing early forms of lymphocytes, believing that certain characteristics might be preserved when using vital stains which might throw some light on the relationship of lymphocytes and their precursors to the cells of leukemia. MacCarty has insisted, that, theoretically at least, the cells of lymphatic leukemia should be lymphoblasts rather than lymphocytes, in a sense regarding leukemia as a malignant condition of the blood comparable to the cells of tissue malignancy. Furthermore, he has demonstrated lymphoblasts in lymphatic leukemia (Fig. 1).

In the routine study of the blood both neutral red and polychrome-

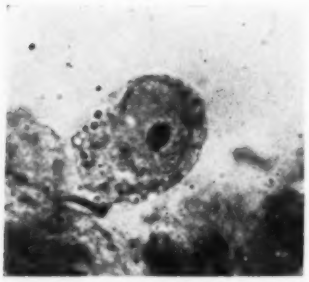

Fig. 3.—Single cancer cell.

Fig. 4.—Myoblast.

methylene blue were used. The latter is preferred because of its application to fresh tissue. Strict cleanliness was used in the preparation of the cover slips and slides, care being taken that no grease or dust adhered. The polychrome-methylene blue was dissolved in Ringer's solution or physiologic salt solution, and a small drop placed on the cover slip together with a small drop of blood, and quickly inverted and placed on the slide, the edges immediately being sealed with vaselin to prevent evaporation. A constant body temperature was maintained by means of an electric stage warmer. In watching the activity of the white cells, attention was directed to the presence of a definite area of clear cytoplasm beyond what is generally considered the periphery of the polymorphonuclear leukocytes. These cells appeared to be living as they were motile, as manifested by the slow protrusion of pseudopods and the rhythmic movement of the granules in the substance surrounding the nuclear bodies. Such activities of the leukocyte continued for several minutes. Cessation of motion was rapidly followed by an increase in the color density of the nuclear bodies, accumulation of granules at the periphery, absence of rhythmic movement, and the sudden bursting of the cell with liberation of the granular contents.

Why this cell layer of cytoplasm was present as a part of certain leukocytes and not of others was difficult to explain. From the study of fixed blood smears numerous investigators have called attention to the discrepancy in the leukocyte count when using Ehrlich's stain and the modern Wright or Balch's stain, the difference in the two readings being from 15 to 20 per cent greater with the triacid stain. When using the Ehrlich stain, the films are fixed with heat, whereas when using Wright's blood stain, the smears are fixed with alcohol. Heating apparently does not destroy the cells which contain the outside layer of cytoplasm as does alcohol.

During my study of the morphology of the blood elements with polychrome-methylene blue, Sabin was studying blood, using the nontoxic stain, neutral red. When her attention was directed to these cells she immediately recognized them as cells which she had also seen and regarded as old polymorphonuclear cells nearing the stage of disintegration, and having a very feeble cell structure.

This type of cell was probably referred to by Schilling, in 1908, who recognized it while studying blood cells with dark-field illumination. At that

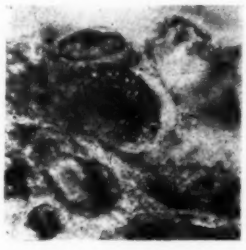

Fig. 5.—Cancer cells.

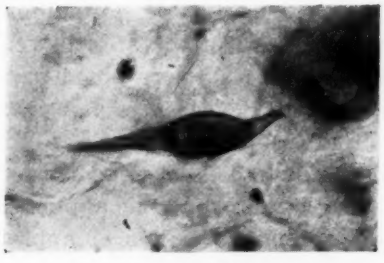

Fig. 6.—Fibroblast.

time he regarded it as a dead leukocyte, and described similar cells in the study of sputum. Since the presentation of this paper, Sabin has published a very complete and instructive study on the living human blood cells with a complete review of the literature, giving further proof of the great possibility of the application of vital stains to the study of living tissue. It seems probable that this type of leukocyte may prove of considerable importance in the life cycle of normal leukocytes and the relationship of disease to their production, possibilities which may be determined by means of the vital stain.

That vital stains have a real place in the study of pathologic secretions can be easily demonstrated. The method has been adopted as a routine procedure in cases of specific urethritis in the Mayo Clinic. Material expressed from the prostate and seminal vesicles is studied in a fresh condition and also in smears. Desquamated epithelium and leukocytes are distinctly stained, and stand out sharply, thereby making it possible to distinguish the different cells and elements. Spermatozoa are clearly stained, and any variations in their structure may be easily noted.

Intracellular diplococci can be distinctly seen, but they are best studied in the smear, using the polychrome-methylene blue stain, which is much

superior to methylene blue and furnishes splendid pictures of cell structure and bacteria.

The nontoxic stains are probably of greatest scientific value. Nevertheless, it is possible that future research with these stains may throw some light on phagocytosis and the influence of dyes, and their antiseptic qualities on the activity of leukocytes. The nontoxic stains differ from the toxic in that cells under the proper temperature remain alive for several hours, whereas the life of the cells exposed to the toxic group is probably more correctly expressed in terms of minutes.

Secretions from the urethra, during acute gonorrheal urethritis, have been studied, using neutral red as a stain. Leukocytes have been observed which were very active, extending their pseudopods in rapid succession, and containing as many as eighteen pairs of intracellular cocci which were stained an orange red. The interesting observations made by Plato that only the intracellular diplococci were stained, while none of the extracellular organisms took the stain, were noted. Even after an exposure of the organisms to the dye for several hours, none of the gonococci outside of the leukocytes were stained.

From the study of normal blood, it seems possible that the study of pathologic blood might afford even more promising and instructive investigation. In places where blood parasites are endemic, the use of vital stains may facilitate the findings and characteristics of malarial parasites, trypanosomes and filaria. Observations of the morphology of blood lymphocytes and leukocytes, pus cells, normal tissue cells and malignant cells, suggest a large field of usefulness in the application of vital stains to the study of living tissue, opening up means of approach for the observation of living cells in their natural habitat.

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KIDNEY INVOLVEMENT FROM PROSTATIC HYPERTROPHY
A NEW THEORY OF EXPLANATION

By A. D. Bush, Emory University, Ga.

ASSOCIATED with prostatic hypertrophy, and more or less sequential thereto are several forms of kidney involvement. One type manifests itself by a decrease of urea eliminated daily, with an increase in the per diem urine. Here the assumption is that there is a lessened absorptive power for water in the tubules, and a diminution therein of urea excretion (if one theory of kidney activity is accepted), or an increased filterability of water, but not of urea, in the glomeruli (if the other theory of kidney activity is accepted). When this condition becomes aggravated there seems to be retention of other excretory products besides urea, with the danger of uremic poisoning impending; the majority of operators consider such cases unjustifiable surgical risks until the kidney functioning shall have been improved by suitable medication.

A second type of kidney involvement is pyelonephritis, amounting, estimates Dr. Bumpus,[1] to "fully 75 per cent of our cases of prostatic hypertrophy." In such cases the remark of Dr. Quimby[2] is pertinent: "Doubtless, also, the kidney is somewhat incapacitated before infection occurs." Probably most clinicians would feel that the pyelonephritis was secondary to infection first elsewhere, cystitis being by far the most common focus. From the bladder the secondary infection of the kidney might conceivably take place by way of the ureter, the blood stream, or the lymphatics.

A third type of kidney involvement in hypertrophy of the prostate is that of necrosis due to back pressure from the bladder through a dilated ureter. Judging by the figures kindly furnished by Dr. Bumpus, this type might amount to about 7 per cent of total cases.

What is the mechanism whereby the kidney's function becomes more or less impaired as a result or concomitant of enlargement of the prostate? There seems to be a rather extensive, though somewhat uncritical opinion that this impairment is due to reflux pressure exerted by a distended bladder through the ureters. We sent a questionnaire to over a score of prominent urologists in the United States, obtaining the courtesy of a reply in about 65 per cent of the queries. Of this number 50 per cent expressed belief in such mechanical causation of the kidney impairment, 42 per cent expressed disbelief, and 8 per cent were noncommittal. Probably all would concede that, in the presence of ureteral incompetency, vesical backpressure would ultimately tend to interfere with kidney functioning; but does such reflux pressure occur in the usual hypertrophy case?

Reflux does not seem to be a normal phenomenon. In his Anatomy, Cun-

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ningham states (3): "The ureters open into the bladder by two small slit-like apertures which are of a valvular nature and prevent a backward passage of fluid into the ureters." This same veiw is also held by physiologists; vide Howell,[4] Starling,[5] Schaeffer,[6] Burton-Opitz,[7] Flack-Hill,[8] Halliburton.[9]

In their text, Kelly and Burnham[10] make the positive statement: "Refluxes do not occur in human beings unless the vesical orifice of the ureter is injured": Kretschmer,[11] however, presents several cystographs showing unilateral reflux in what he claims are normal children; but a critical study of the pictures shown throws much doubt on the assumed normality. All the bladder shadows are of unusual contour, two of them markedly so; and none of them shows bilateral reflux. It might seem more probable that these cases are examples of developmental insufficiency than of normals showing unphysiologic departures; yet the mere fact of the finding of such developmental inadequacy indicates a probable explanation for whatever cases of hydrouretos, of distal initiation, later may be found.

Some experiments have been performed to test the efficiency of the valve-like openings of the ureters into the bladder to resist varying intravesical pressures. Lucas[12] reports ten experiments where intravesical air pressures, gradually increased to the point of rupture of the bladder, showed no reflux into the ureters. Twelve experiments recently performed on dogs, in my laboratory, showed no reflux into the ureters even when intravesical pressures were as high as 120 centimeters of water. True, both Lucas' experiments and mine were performed on dogs; but as Kelly and Burnham[13] state: "The lower end of the ureter (in dogs) strongly suggests the arrangement found in man."

It seems fairly reasonable to conclude, then, that reflux is not a normal physiologic condition; but does it occur in hypertrophy of the prostate, and is the impairment of the kidney thus produced? Dr. Bumpus[1] states: "We see urea retention in the vast majority of our prostatic cases when no reflux exists." Dr. Deaver[14] states the customary mechanical theory, then adds: "I do not think this same sequence of events has been proved to exist in the diseased prostate, though it is a plausible and most convenient theory."

Those who do not accept the theory of reflux seem inclined to feel, nevertheless, that the kidney impairment is largely due to a mechanical back pressure up the ureter; they explain this as due to the increasing difficulty of the ureter to expel its load into a bladder having high intravesical pressures. This theory may well be examined in the light of experiment and observation.

Campbell[15] made about twenty observations in the Urological Service of Bellevue Hospital. He connected a catheter through a three-way stop-cock with a mercury manometer so as to study intravesical pressures (in acute prostatic retention), and the variations produced by fractional withdrawals. He found intravesical pressures to range from zero, in atonic conditions, to a maximum of 30 mm. Hg.; and that the withdrawal of but small amounts markedly reduced the prevailing pressure.

Burton-Opitz[16] states: "Under ordinary conditions, micturition does not result until the pressure in the bladder has risen to about 150 mm. water"

(equal to about 11 mm. Hg.). But Lucas[17] has shown that the tonicity of the ureter is not normally overcome until about 60 cm. water (equal to about 45 mm. Hg.) pressure has been exerted; and (on page 251) he records obtaining tracings from an isolated piece from the middle portion of a dog's ureter which for forty minutes contracted regularly four or five times a minute against a pressure of 86 cm. Ringer solution (equal to about 63 mm. Hg.). In this connection it is interesting to note that the secretion pressure of the kidney is stated by Starling[18] to be about 40 to 50 mm. Hg. less than arterial pressure, that is, normally at least 60 mm. Hg.

From these observations it would seem that the theory of the inadequacy of ureteral contractions to meet intravesical pressures, with a sequent damming back of pressures into the pelvis of the kidney, could not well be maintained, unless the assumption be made that the ureteral wall is undergoing a gradual process of detonization as a result of the trigonal alterations; it is not improbable that this secondary effect on the ureter may sometimes occur. But seemingly a more probable theory of accounting for the kidney involvement can be derived from the facts of nerve interrelationship.

From our studies of embryology it will be recalled that the ureter, the pelvis of the kidney, and the collecting tubules of the kidney, originate from the posterior surface of the Wolffian duct just proximal to the point where that duct empties into the cloaca; and that the balance of the kidney apparently separates off from the blastema of the adjoining Wolffian body, and very early encaps the outpushing bud forming the anlegal ureter. That part of the urogenital sinus, just distal to the point from which the ureter buds forth, later becomes the trigone of the bladder; embryologically it is continuous with the part from which the ureters, pelves of the kidneys, and the collecting tubules, are formed, and immediately adjacent to the part from which the balance of the kidneys is formed (McMurrich[19]). The nerve supply to the trigonal area of the bladder is by way of the hypogastrics, through the aortic plexus, from the eleventh and twelfth thoracics and the first lumbar (Schaeffer[20]); while the supply to the kidneys is largely from the tenth, eleventh, and twelfth thoracics (Cunningham[21]). Wilder,[22] claims that "The nerve which originally supplies a given primitive element * * * never forsakes it, but follows it through all its vicissitudes and continues to supply its derivatives, of whatever complexity or form, through all the changes of relation." And here exists a continuous structure consisting of kidney, pelvis, ureter and trigone, developed from contiguous parts of the caudal portion of the Wolffian body, and supplied by nerves arising in major part from the same lower thoracic segments, so, because of the kinship of embryologic origin, and the persisting neurologic connections, a functional interrelationship may reasonably be postulated. Enlargement and irritability of the prostate produce sequent alterations in the trigone; the close relation of the trigone with the kidney, both embryologically and neurally, may reasonably result in sequent alterations in the kidney.

The contentions advanced in the preceding paragraph are further buttressed by clinical data, with the anatomic and physiologic explanations advanced therefor.

Piersol[23] states: "The same segments of the spinal cord supply the abdominal viscera as the skin and muscles over them"; and Schaeffer[24] adds: "Referred pain (and tenderness) will occur in the skin areas of those roots which have white rami." We assume that the visceral afferents, under normal conditions, carry impulses having to do with functional reflex activity, impulses that do not rise to the level of consciousness; but under certain pathologic conditions the afferent impulses may be so summated that they produce such an altered or heightened sensitivity in the dorsal ganglia as to induce afferent impulses in correlated cutaneous nerves; such induced sensations from skin areas constitute most "referred pain." Schaeffer[25] states: "Disease of the kidney and ureter cause reference of pain and hyperalgesia in the skin the sensory innervation of which is from the tenth, eleventh, and twelfth thoracic spinal ganglia." Pottenger[26] illustrates the chief areas involved in renal viscerosensory reflexes, as well as in the viscerotrophic reflexes in chronic kidney diseases, as a zone encircling the torso just below the umbilicus; and on page 262 he presents another illustration indicating a closely similar area for those prostatic viscerosensory reflexes mediated though the hypogastric nerves.

But referred pains may be to some viscus of close nerve relationship. For instance, Kelly and Burnham[27] state: (In some cases of tuberculosis of the kidney) "Marked vesical distress is met with where cystoscopic examinations show a healthy-looking bladder." Of course, the irritation may be due to urine changes, in some cases; in other cases, it is presumably a referred pain. Such assumption is supported by consideration of the symptoms frequently noted in renal and ureteral calculus; for example: "Vesical irritability, in some cases amounting to strangury, may occur with kidney and ureteral stones without there being any disease of the bladder itself. * * * It is practically as common when the stone is in the kidney as when it is in the ureter. During the attacks of colic it may be quite severe. We observed it in 40 per cent of the ureteral cases, and in 37 per cent of the kidney cases. In cases where no infection was present, 45 per cent showed more or less vesical distress during the attacks, and sometime during the intervals. * * * In some cases in place of irritation and strangury there was simply pain in the bladder." (Ibid. 141.)

Proceeding down the ureter, it is found that: "Pressure upon the ureter (during an examination) is almost invariably followed by pain and an intense desire to empty the bladder[28]"; also, reversely, "both polyuria and threatened suppression have followed the gentle and partial insertion of the ureteral catheter.[29]" It seems that the nearer the ureteral stone, or the irritation, is to the vesical outlet of the ureter, the more marked are the vesical symptoms of irritability, frequent urination, and tenesmus. Concerning the several referred pains, Piersol[30] remarks: "The relations of the nerve-supply of the ureter with that of the bladder * * * with the great intraabdominal plexuses sufficiently explain the chief subjective symptoms of calculus."

Next consider the trigonal region of the bladder, with its underlying and closely adherent prostate. Says Piersol[31]: "The mucosa of the bladder, supplied by the hypogastric plexus, is not very sensitive normally, except in

the region of the trigonum. There it is tightly connected with the muscular layer; and the loose elastic submucous connective tissue found in the remainder of the bladder is absent. * * * This close adhesion of the mucous and muscular layers prevents free swelling when inflammation occurs, and, in conjunction with the particularly generous vascular and nerve supply to the trigonum and neck of the bladder, explains the pain and sensitiveness of that region in cystitis," or other conditions producing undue tensions in that area. The nerve supply to the prostate is chiefly from the hypogastric, as is that of the trigonum; so it can easily be appreciated, as Piersol states (ibid): "Other referred pains in vesical disease are to the lumbosacral region * * * —through the hypogastric plexus; (and) to the kidney, by the junction in the spermatic plexus of filaments from the vesical and renal plexuses." In acute prostatitis "referred pains are very common, and, on account of the derivation of the nerve supply of the prostate from the lower three dorsal * * * segments, are apt to be widely distributed, as e.g., pain over the tip of the last rib" (ibid, 1980). In hypertrophy of the prostate two sets of the several groups of symptoms outlined by Piersol are: "Frequent urination, due partly to the inability completely to empty the bladder, but chiefly to the venous congestion about the trigonum," and "referred pains similar to those noted as occurring in acute prostatic swelling" (ibid, 1982). In this connection it should be noted that a circulatory disturbance results from the pressure which the enlarging prostate exerts on the veins into which the vesical veins empty, and this in turn induces a vesical irritability which gives a vicious circle of heightened passive hyperemia which of itself tends to develop an "erethism more potent than residual urine to occasion the frequently recurring desire to urinate" (Piersol, ibid, 1981). It may be, too, that this hyperemia aids in the absorption of toxic products from residual urine, and thus throws additional work on the irritated kidneys.*

EVIDENCE FROM EXPERIMENTATION

In 1889 Bradford[32] found that stimulation of the nerves from the 4th thoracic to the 4th lumbar produced vasoconstriction of the kidneys, with maximum effect produced from stimulation of the eleventh and twelfth thoracic; and Schaeffer[33] reports that he has "seen in the cat prompt and great pallor of the kidney and of the upper part of the ureter on stimulating the first lumbar nerve." Stimulation of the distal portion of the hypogastric nerve causes, among other things, "a weak contraction of the bladder, chiefly in the region around the ureters, but little or no contraction of the blood vessels" (ibid. 646). A similar statement is made by Starling[34]: "In the dog (stimulation of) the hypogastric nerves cause a strong contraction of the muscle fibers at the base of the bladder, especially of the trigonum."

Schaeffer[35] states: "About one-tenth of the medullated fibers in the hypogastric nerves are afferent"; and of course impulses traveling from the peripheral distribution of this nerve must eventuate in various reflexes; do any of these impulses get switched to the kidneys, and, if so, what results?

(*We shall publish findings soon that show definite absorption of drugs by the vesical mucosa.)

Not finding answers to these questions in the literature, we put them to the experimental test.

The methods of our experiments, details of which will later be published, consisted, in one set of experiments of stimulating the proximal end of the cut hypogastric and noting the variations in oncometer records, and in the other set of experiments in noting the relative secretion of urine from the normal kidney as compared with that from the kidney on the side where the proximal end of the cut hypogastric was being stimulated. The first set of experiments gave inconstant results, the tendency being toward slight vasoconstriction. The second set of experiments required many tedious attempts, as anuria occurred in 75 per cent of the dogs operated on; but the successful experiments showed invariably a marked increase of urine secretion following minimal stimulation with the interrupted faradic current (two seconds of stimulation every ten seconds). Further experiments are being conducted to note what change, if any, occurs in the quality of the secretion when thus increased.

These results seem to indicate that afferent impulses along the hypogastric may alter the activity of the kidney. Long-continued irritation of the trigone area, brought about by passive hyperemia, residual urines, and vesical tenesmus, would inevitably result in a summation of afferent impulses. In some cases these afferent impulses might eventuate in referred pains in the cutaneous areas supplied by the eleventh and twelfth dorsals and first lumbar; such, indeed, as are frequently found clinically. In yet other cases it seems reasonable to conclude that such summation of afferent impulses might so alter kidney activity as to result in the clinical manifestations encountered occasionally in hypertrophy of the prostate.

Deaver states[36]: "Only about one person in every seven who has an enlarged prostate suffers from it." A small number of the total have kidney involvement directly due to back pressure from the bladder; a sequence that would seem to be preceded, necessarily, by a marked diminution of ureteral tonicity. A large number seem to have a sequent pyonephrosis, presumably following an infective cystitis. This group, and still another group having polyuria and other evidences of kidney involvement (but noninfective) are probably undergoing neural reflexes arising at the trigonal area. Of course, from these several groups must be excluded those prostatics who are at the same time suffering from arteriosclerosis, with accompanying high blood pressure and chronic Bright's disease, whose general condition is cardiovascular, primarily, and not renal.

NOTE. Since the above was written Graves and Davidoff[37] have published a series of investigations made upon rabbits; they found regurgitation took place in 73 per cent of normal cases, and in 78 per cent of operative cases. These findings, however, must be viewed with great caution when we think of transferring them to man. It is well known that the arrangement of muscle fibers in the bladder of the rabbit is much less like that of man than is the arrangement of muscle fibers in the bladder of the dog. So while

the dog findings may reasonably be transferred to man,[38] findings with the rabbit must be weighed with more reservation.

SUMMARY

1. A rather widespread opinion exists that kidney involvement accompanying prostatic hypertrophy is due to back pressure from the bladder through the ureters.

2. Physiologists very generally deny the possibility of such a reflux under normal conditions.

3. Experiments on dogs (whose vesical architecture is very much like that of man), with the object of obtaining a reflux if reasonably probable, yielded no refluxes even under excessive intravesical pressures.

4. Embryological studies show a closely related origin of nephritic tissue and vesical trigone.

5. Clinical data show a closely coordinated innervation of kidney, ureter, and trigone, as indicated by the similarity of referred tenderness and pain in acute congestions and inflammations of these several viscera. The close adhesion of the trigonal tissue to the underlying prostate involves this portion of the bladder in disorders of the prostate.

6. Experimental data indicate that electric stimulation of the proximal end of the cut hypogastric nerve affects the functional activity of the kidney.

7. From these several findings the conclusion is drawn that uncomplicated kidney dysfunction, accompanying prostatic hypertrophy, is a condition primarily due to neural reflexes coming from the area of the trigone by way of the hypogastric afferents and then out through the eleventh and twelfth thoracic autonomics to the kidneys.

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APPLICATION TO THE CEREBROSPINAL FLUID OF THE COMPLEMENT-FIXATION TEST FOR GONORRHEA IN OBSCURE CASES OF NERVOUS DISEASE*

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IT has been known for a long time that the gonococcus of Neisser may attack the central nervous system (in this connection see textbooks on venereal diseases generally and especially the textbook of Luys[1]). Connor[2] has recently passed in review all cases mentioned in the previous literature and has revealed the apparent fact that gonorrhea may give rise to every single pathologic affection of the nervous system from psychoses of various types to pseudotabes, cerebrospinal meningitis, myelitis, polyneuritis, neuralgias, and so forth. The symptomatology of most of these cases, the history of few of which he reports in full, makes one think at first of a luetic infection, which supposition is, however, excluded by the history and by the fact of negative Wassermann reaction on the blood serum on the cerebrospinal fluid.

Individual cases of nervous lesion traceable to gonorrhea appear now and then in the literature, and only last year Brunn[3] in Vienna gave a demonstration of a case of gonorrheal hemiplegia in which with a Wassermann-negative serum, the gonococcus was isolated from the blood of the patient. As the gonococcus may also be isolated from the joints in cases of gonorrheal arthritis and as the blood of patients gives the complement-fixation reaction, both in cases of urethral and of arthritic gonorrhea, we must conclude that the disease is a systemic one. Now, the gonococcus is universally conceded to be very similar to meningococcus,[4] and there is even the possibility that they may represent phases of one and the same organism.[5] As the greater number of pathogenic microorganisms including the meningococcus have been found to attack the nervous system in one way or another, one should be prepared to recognize that the gonococcus may equally be a culprit.

In his book on the cerebrospinal fluid Boyd[6] mentions as causes of cerebrospinal meningitis the meningococcus, the pneumococcus, streptococcus,

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tubercle bacillus, staphylococcus, typhoid bacillus, bacillus coli, influenza bacillus, bacillus pyocyaneus, anthrax bacillus, Friedländer pneumobacillus, bacillus mallei, actinomyces, streptothrix, Spirocheta pallida, trypanosoma, and Diplococcus rheumaticus, but makes no mention of the possibility of a gonococcus infection. In another part of his book he speaks of the meningococcus as "the sole and sufficient cause of cerebrospinal meningitis," adding, "it is at least certain that different strains of meningococcus exist." In view of the astonishing similarity in biological and cultural reactions between the meningococcus and the gonococcus there is always the possibility that they may represent different phases of one and the same microorganism.

Müller and Oppenheim[7] were the first to apply the complement-fixation test for gonorrhea, and today the specificity of the reaction is unanimously accepted as yielding from 80 to 100 per cent of positive reactions in case of gonorrheal urethritis. At the same time the routine practice of this reaction is not customary, clinicians as a rule being satisfied with a history of urethral discharge and with the presence therein of the gonococcus, having recourse to the complement-fixation test for gonorrhea only in cases of obscure arthritis.

As the literature, as far as I know, is mute in regard to the reaction of the cerebrospinal fluid to the complement-fixation test in cases of gonorrhea, I started a year ago a series of investigations on the subject, and was interested to find that quite a number of cerebrospinal fluids examined gave a positive reaction. Some cases had symptoms of pseudotabes with spastic gait and marked mental disturbances suggesting syphilitic infection, the Wassermann reaction for lues both on the serum and the cerebrospinal fluid being, however, negative. This result led me to enquire very carefully into the clinical history of patients from whom the Wassermann-negative and gonorrhea-positive cerebrospinal fluids had been obtained.

While no history of lues was forthcoming, a history of chronic gonorrhea was in each case elicited. In one particular case, viz., of facial paralysis, the Wassermann reaction both of the blood and of the spinal fluid was negative. Inquiry in this instance revealed no history of lues but a definite history of chronic gonorrhea. In another case, one of transverse myelitis, there was again no history of lues and the Wassermann test was negative toward the blood and the spinal fluid. In still other cases in which the diagnosis of lues could be excluded clinically and biologically I obtained a strong positive complement fixation for gonorrhea in all dilutions, while inquiry into the history revealed a previous gonorrhea. As the present paper is concerned simply with the biological test I will reserve for later report the clinical data.

Technic.—I have adopted the technic given by Kolmer,[8] point by point, using all the care necessary for carrying out such a delicate reaction.

A few remarks remain to be made regarding the behavior of the cerebrospinal fluid obtained from cases of gonorrhea of the nervous system.

1. As a rule blood serum and cerebrospinal fluid give parallel results, though often the cerebrospinal fluid may give a strong positive complement-

fixation reaction for gonorrhea while the same reaction on the blood serum may be negative.

2. The cell count and the globulin of the cerebrospinal fluid may remain normal or slightly increased, notwithstanding a positive complement fixation for gonorrhea.

3. The colloidal gold reaction of Lange and the colloidal mastic test of Emanuel apparently do not seem to be affected by the positivity of the cerebrospinal fluid for gonorrhea, though often one notices a slight reduction in the mid zone, but only in the case of the colloidal gold reaction.

4. A cerebrospinal fluid may naturally be positive to lues and to gonorrhea, if the two infections are present.

5. The gonococcus may apparently be present in cerebrospinal fluid, for I have often seen a gram-negative diplococcus arranged in pairs like the gonococcus. Identity however was not established by culture.

In conclusion we may say that while the number of cases examined is as yet too small to permit one categorically to assert that the method of diagnosis is infallible, nevertheless the inference is warranted that we have in the complement-fixation test as applied to the cerebrospinal fluid a definite means of diagnosing gonorrhea of the nervous system. It is further probable that the same method can be applied in the case of other microorganisms which invade the cerebrospinal system.

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THE URIC ACID CONTENT OF SPINAL FLUID*

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IN a previous communication,[1] a study of the uric acid in spinal fluid was presented in a few cases. This paper completes the study in a larger number of cases.

The first complete work on the nonprotein nitrogenous constituents of spinal fluid of children was reported by Leopold and Bernhard[2] in 1917. They were unable to demonstrate uric acid quantitatively in normal spinal fluids, found only traces in nephritics, and measureable amounts in the cases of tuberculous meningitis examined by them. Myers and Fine[3] reported a series of fifteen cases of nephritis, the uric acid varying from 0 to 1.5 mg. per 100 c.c. of fluid. Thirteen showed an increase of all nonprotein nitrogenous constituents. They concluded that the uric acid concentration of the spinal fluid was about five per cent of that found in the blood. Kahn and Neal[4] however, report findings in which the uric acid was absent or present only in minute quantities.

All of the above workers were inclined to attribute the absence of uric acid in the spinal fluid to the fact that it does not pass readily through the choroid plexus into the spinal fluid of normal individuals. The method generally used was either the older method of Folin and Denis,[5] or Benedict's[6] modification of this method, involving the use of silver magnesia mixture for the precipitation of the uric acid. In low concentrations of uric acid this method requires rather large amounts of material, and the precipitation of uric acid is not quantitative when small amounts are present. These difficulties have been overcome by Benedict,[7] who has devised a direct method of estimating uric acid without any previous precipitation and utilizing a reagent which is specific for this substance, and is exceedingly accurate when small amounts are present. This method has been used exclusively both for the the spinal fluid and blood examinations reported in this paper.

Method. To 2.5 or 5 c.c. of spinal fluid is added distilled water to 10 c.c., 4 c.c. of 5 per cent sodium cyanide and 1 c.c. of Benedict's arsenic tungstic acid reagent. The mixture is placed in boiling water-bath three minutes, and after cooling three minutes, it is compared in the colorimeter (Dubosecq) with a standard solution of uric acid (5 c.c. = 0.02 mg. uric acid), to which has been added distilled water to 10 c.c. and the same amounts of sodium cyanide and reagent, and treated in the same manner as the spinal fluid. It is convenient to set the standard at 15. The colors obtained are very easy to compare. If R represents the reading of the standard, S the strength of the standard, and Y the reading of the unknown, the calculation will be:

$$\frac{R \times S}{Y} = \text{Milligrams of uric acid in the amount of spinal fluid used.}$$

*From the Pathological Laboratory of the Lenox Hill Hospital, New York.
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TABLE I

URIC-ACID CONTENT OF SPINAL FLUID

HIST. NO.	AGE	SEX	URIC ACID MGS. PER 100 C.C.	BLOOD U. A. MGS. PER 100 C. C.	PER CENT CONC. OF BLOOD	SUGAR PER CENT	GLOBULIN	CELLS	DIAGNOSIS REMARKS
7203	9	M	0.62	–	–	0.051	–	–	Chorea
7447	12	M	1.00	–	–	0.065	–	–	Chorea
8025	9	F	0.44	2.5	17.6	0.055	–	–	Chorea
8785	12	M	0.72	3.4	21.2	0.071	–	–	Chorea
353	10	M	1.10	4.0	27.5	0.065	–	–	Chorea
891	9	F	1.00	4.2	23.8	0.066	–	–	Chorea
721	12	F	0.80	4.0	20.0	0.080	–	–	Chorea
998	11	F	1.00	3.1	32.2	0.078	–	–	Chorea
1033	10	F	0.60	3.2	18.7	0.080	–	–	Chorea
1169	8	F	0.66	–	–	0.068	–	–	Chorea
1128	9½	M	0.60	–	–	0.060	0	0	Chorea
1006	11	F	0.74	3.5	21.1	0.080	–	–	Chorea
2054	7	F	0.40	2.7	14.8	0.060	–	–	Chorea
1431	8	F	0.92	4.4	20.9	0.088	0	5	Chorea
1745	11	F	1.32	3.7	35.6	0.083	0	12	Chorea
495	21	M	0.88	3.1	28.4	0.062	0	0	Acute Polio Encephalitis
590	60	M	0.80	5.2	15.3	0.100	0	26	Encephalitis Lethargica
679	42	F	0.92	3.8	24.2	0.075	0	0	Encephalitis Lethargica Acute Polio Encephalitis
8519	40	M	2.0	4.3	46.5	0.030	2+	820	Died. Diagnosis?
9103	8	F	1.20	3.5	34.2	0.062	0	0	Encephalitis Lethargica
466	9½	M	0.65	4.2	13.0	0.057	0	0	Encephalitis Lethargica
529	29	M	0.50	3.0	16.6	0.051	0	0	Encephalitis Lethargica
839	47	M	0.36	–	–	0.085	–	–	Tabes Dorsalis
7339	67	F	0.30	–	–	–	0	40	Syphilis C. S.
8198	44	M	0.43	4.8	8.9	0.062	0	30	Syphilis C. S.
8874	43	M	0.46	–	–	0.062	–	0	Syphilis C. S.
489	27	M	0.46	3.0	15.3	0.055	0	10	Syphilis C. S.
336	69	F	0.60	4.8	12.5	0.076	–	–	Chronic Nephritis Uremia. Died
1879	27	M	3.1	8.5	36.4	0.160	0	5	Sp. Fld. Urea N. 33 mgs.; creatinine 5.5 mgs.; Blood Urea N. 143 mgs.; creatinine 14.4 mgs.
.1	2 mos.	M	1.48	–	–	0.055	0	10	Congenital Hydrocephalus
1218	7	F	0.55	3.1	16.7	0.088	0	0	Lobar Pneumonia
567	51	F	0.26	2.4	10.8	0.088	0	0	Arteriosclerosis
817	29	M	0.80	4.6	17.3	0.080	0	0	Chronic Orchitis
1641	1	M	0.36	2.7	13.3	0.088	2+	250	Bronchopneumonia
1721	9	F	1.0	2.4	41.6	0.071	0	10	Hemorrhage into Cerebrum
2109	1¾	M	0.55	1.7	32.1	0.080	–	–	Infantile Convulsions
1973	6	M	0.46	3.2	14.3	0.090	–	–	Concussion of Brain Fracture of Skull
P303	24	M	0.40	–	–	0 083	0	0	Concussion, Coma
7666	5	F	0.62	2.4	25.8	0.083	±	10	Acute Poliomyelitis
9008	13	M	0.30	–	–	0.112	0	10	Sinus Thrombosis Double Mastoid
8575	11	F	1.00	–	–	0.083	0	0	Mastoid
126	8	M	0.80	–	–	0 055	+	4600	Ac. Meningitis
348	3 mos.	M	0.35	–	–	0.076	–	20	Hemorrhage into Brain
395	52	M	0.60	4.2	14.2	0.066	0	0	Influenza
8626	41	M	0.42	3.0	14.0	0.120	0	0	Chronic Arthritis

TABLE I—CONT'D

URIC-ACID CONTENT OF SPINAL FLUID

HIST. NO.	AGE	SEX	URIC ACID MGS. PER 100 C.C.	BLOOD U. A. MGS. PER 100 C. C.	PER CENT CONC. OF BLOOD	SUGAR PER CENT	GLOBULIN	CELLS	DIAGNOSIS
7953	1¾	M	4.80	–	–	0.020	2+	250	Tuberculous Meningitis
150	2	M	3.50	–	–	0.00	4+	16,000	Meningococcus Meningitis
537	31	F	1.40	3.0	46.6	0.020	4+	2,200	Suppurative Cerebral Pachymeningitis
573	8	M	1.10	2.0	55.0	0.030	2+	260	Tuberculous Meningitis
1383	2	M	1.80	1.6	62.5	0.00	4+	5,000	Meningococcus Meningitis
869	34	M	0.46	3.5	13.1	0.065	±	0	Suppurative Pachymeningitis

Discussion. Table I records the uric acid, sugar content, as well as other data in fifty-one cases. The uric-acid content varies from 0.26 mg. (case of arteriosclerosis) to 4.8 mg. (case of tuberculous meningitis). Thirty-two of the cases examined were under thirteen years of age (severe nephritides or infections where high uric-acid figures are obtained, are left out of the averages), the uric acid varies from 0.35 to 1.48 mg., the average being 0.76 for children. In adults, which includes sixteen cases, the uric acid varies from 0.26 to 0.92, the average being 0.54 mg., which is considerably lower than that in children.

The uric acid in fifteen cases of chorea varied from 0.40 to 1.32 mg., the average being 0.78 mg. This is in contrast to the twenty-one cases reported by Leopold and Bernhard,[2] in which they were unable to demonstrate any uric acid, by the older methods for its estimation.

In the six cases of bacterial infection, all of whom died, recorded in Table II, every one showed an increase of uric acid, while the sugar content as determined by the method of Folin and Wu[8] was greatly reduced. The series reported by Leopold and Bernhard[2] showed a similar relationship, although the uric acid could not be demonstrated quantitatively in every case. The question arises here whether the increased uric acid in these cases might not be accounted for by the great increase of cells. From an examination of the data in Table I it will be seen that Case No. 126 with 4600 cells had a uric-acid content of 0.80 mg., Case No. 1641 with a cell count of 250 had a uric acid of 0.36 mg., while in Case No. 1 with a uric acid of 1.48 mg., the cell count was only 10; and Case No. 1879 with a cell count of 5, had a uric-acid content of 3.1 mg.

Seven cases of encephalitis showed uric acid which varied from 0.65 to 2.0 mg., the one having the latter high figure terminating fatally.

In the two cases of nephritis reported, the one in which uremia was present showed a marked increase of uric acid (3.1 mg.), the other nonprotein nitrogenous constituents being also increased.

Five cases of syphilis including one case of tabes dorsalis, showed the uric acid to be fairly constant, varying from .930 to 0.46 mg., the average being 0.40 mg.

In all the other cases not mentioned in the above groups, the uric acid varied from 0.30 to 1.0 mg., with the exception of a case of congenital hydrocephalus, in which the uric acid was 1.48 mg.

Relationship of the Blood Uric Acid to That of the Spinal Fluid. In thirty-six cases the blood uric acid was determined simultaneously with that of the spinal fluid. The normal uric acid of the blood as estimated by the new procedure of Benedict is about three milligrams per one hundred c.c. of blood. In the series reported in this paper, the blood uric acid varied from 1.6 to 8.5 mg., the latter figure being obtained in a case of uremia; excluding this figure in the calculations, the average blood uric acid for this group is

TABLE II

URIC ACID IN SEVERE INFECTIONS

HIST. NO.	URIC ACID MGS. PER 100 C.C.	SUGAR PER CENT	CELLS	GLOBULIN	DIAGNOSIS
7953	4.8	0.020	250	++	Tuberculous Meningitis. Died
8519	2.0	0.030	820	++	Acute Polioencephalitis. Died
150	3.5	000	16000	++++	Meningococcus Meningitis. Died
537	1.4	0.020	2200	++++	Suppurative Cerebral Pachymeningitis. Died
573	1.1	0.030	260	++	Tuberculous Meningitis. Died
1383	1.8	000	5000	++++	Meningococcus Meningitis. Died

3.3 mg. The average of all the spinal fluid uric-acid figures excepting the severe infections and the case of uremia is 0.73 mg. It follows that the concentration of uric acid in the spinal fluid would be about 22.1 per cent of that of the blood in the same cases, as against the averaged figure of 5 per cent as reported by Myers and Fine.[3] A study of Table I will show that the uric-acid concentration of spinal fluid as compared with that of the blood varies from 8.9 per cent to 62.5, and is due, no doubt, to the great number of different conditions studied.

In chorea, the uric-acid concentration of the spinal fluid as compared with that of the blood varied from 14.8 per cent to 35.6 per cent, the average being 23.0 per cent, while in encephalitis, it was 23.8 per cent. It is obvious that any increase of the spinal fluid uric acid, especially in infections, would cause a rise in the ratio to the blood concentration.

SUMMARY

The uric-acid content of the spinal fluid has been determined in fifty-one cases. In children the average uric acid was 0.78, while in adults, the average was 0.54 mg. per 100 c.c. fluid.

In chorea, the average uric acid was 0.78 mg. The sugar content was within normal limits.

Syphilitics showed an average uric acid of 0.40 mg.

In uremia, the uric acid as well as the other nonprotein nitrogenous constituents were markedly increased.

In one case of congenital hydrocephalus, the uric acid was 1.48 mg.

All cases of severe infection showed an increase in the uric-acid content, while the sugar was decreased.

The average concentration of uric acid in thirty-five cases was 22.1 per cent of the blood concentration.

CONCLUSIONS

1. The uric-acid content of the spinal fluid of children is higher than that of adults.

2. In severe infections the uric acid is greatly increased while the sugar is decreased.

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THE EFFECT OF HYDROGEN-ION CONCENTRATION ON THE PRECIPITATION OF COLLOIDAL BENZOIN AND GOLD SOLUTIONS BY CEREBROSPINAL FLUID*

By Loren W. Shaffer, M.D., Rochester, Minn.

SINCE the introduction of the colloidal gold reaction by Lange, in 1912, a voluminous literature has arisen covering the methods of preparation of the reagent, the clinical application of the reaction and the factors concerned in precipitation. Lange believed that the reactions were caused by the presence in the cerebrospinal fluid of various proteins. Jaeger and Goldstein regarded the reactions as dependent on the electrical changes in the fluid and a purely physical change. Weston's experiments appeared to show that the Wassermann-producing substance and the gold-precipitating substance were different in that the former was not dialyzable while the latter dialyzed freely. Cruickshank definitely found that dialysis did not diminish the capacity of cerebrospinal fluid to precipitate colloidal gold. Felton asserted that the reactions were dependent on the relative amounts of globulin and albumin present; the globulin precipitating and the albumin exercising a protective action on colloidal gold. He also asserted that the reaction in syphilitic diseases of the central nervous system depends on a physical change in the globulin, associated with an increased positive electric charge.

Of the various methods of preparation of colloidal gold, the original

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one of Lange, based on the work of Zsigmondi, has been modified in many ways. The most commonly used are those of Miller, Brush, Hammers and Felton, and of Black, Rosenberg and McBride. It was early recognized that the acidity or alkalinity of colloidal gold played an important part; frankly acid gold sol giving precipitation with normal spinal fluids while alkaline gold sol was not sensitive to paretic fluids.

Mellanby and Davies, in a recent article, describe a new and simple method of preparing colloidal gold solution, and emphasize the importance of its reaction. They express the reaction of the gold solution in terms of normal acid or alkali. I believed that it would be of interest to determine variations in its sensitiveness in terms of hydrogen-ion concentration, and with this as a starting point, the present work was carried out. The method of preparation of gold sol employed by Mellanby and Davies will be found in their original article. It consists of simply adding 1 c.c. of a 1 per cent solution of neutral potassium oxalate to 100 c.c. of distilled water, and heating to boiling. While boiling, 1 c.c. of a 1 per cent gold chloride solution is added drop by drop. Within half a minute a beautiful red solution of colloidal gold is obtained. This method has been used in the work herein reported with satisfaction. Its simplicity is especially valuable when fresh solutions in small quantities are needed.

Owing to the fact that it is difficult to determine slight variations in hydrogen-ion concentration of colloidal gold by the colorimetric method because of color interference, it was decided to run parallel determinations with colloidal benzoin.

The colloidal benzoin precipitation reaction with cerebrospinal fluid introduced by Guillain, Laroche and Lechelle, in 1920, has been widely used as a substitute for the colloidal gold test. Its method of preparation and the technic of the test are well described in the literature. The modification recently advised by Wright and Kermack which consists in heating the distilled water to 55° C. before adding the alcoholic extract of benzoin and filtering, has been used in this work. A more concentrated filtrate is thus obtained, which is, however slightly less sensitive to precipitation. The results appear to be more constant than by the original method, and the solution more stable.

The determinations of hydrogen-ion concentration in these studies were made by the colorimetric method, using the Clark-Lubs phenolsulphonephthalein series of indicators and alizarin red. Although the determinations are not so accurate as those obtained by the electrometric method, they are approximately correct and offer rapid and simple means for hydrogen-ion concentration determination. A number of tests were made and each series of determinations averaged. The readings on colloidal gold were especially difficult and were only attempted within the range of brom-cresol-purple and brom-thymol-blue. In the case of colloidal benzoin the presence of the sol interfered somewhat with the readings in the tubes in which it was not precipitated. By the use of the "comparator" of Hürwitz, Mayer and Ostenberg (1915) readings were made which are believed to be reasonably accurate.

FACTORS DETERMINING THE HYDROGEN-ION CONCENTRATION IN THE INDIVIDUAL TUBES OF EITHER THE GOLD SOL OR COLLOIDAL BENZOIN REACTIONS

Reactions of the coilloidal solutions.—Colloidal gold, according to the accepted standards, should be adjusted to a red color with a distinct tinge of brown, using 1 per cent solution of alizarin red in 50 per cent alcohol as an indicator. This gives a hydrogen-ion concentration of approximately pH 6.4.

Colloidal benzoin solution is definitely acid, probably owing to the presence of benzoic acid. Its average hydrogen-ion concentration in our experience has been about pH 4.2. It is subject to slight variations, depending on the acidity of the distilled water and purity of the gum benzoin. In-

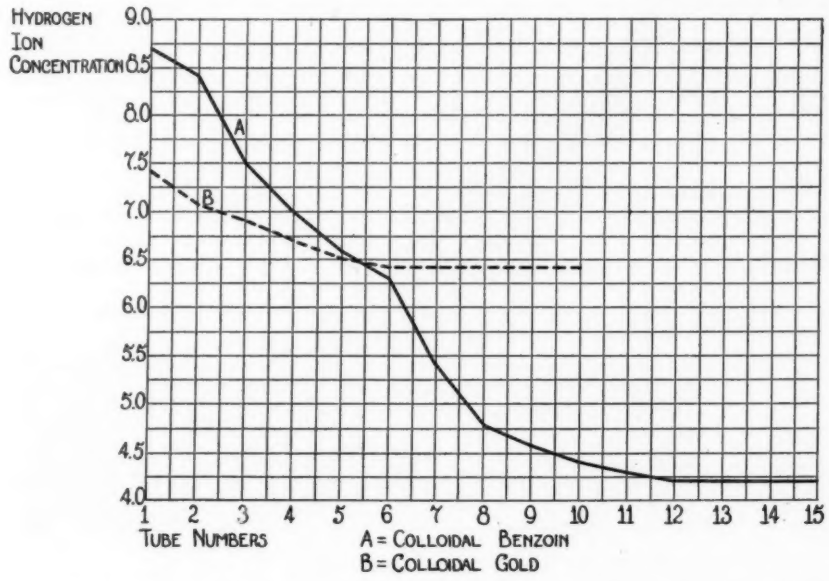

Chart 1.—Curves of hydrogen-ion concentration in the individual tubes of the colloidal benzoin and gold reaction.

vestigators have not considered it necessary to adjust its hydrogen-ion concentration.

Reaction of the cerebrospinal fluid.—Felton has shown that the average hydrogen-ion concentration of freshly drawn cerebrospinal fluid is about pH 7.75, but that it rapidly becomes more alkaline on standing, and at the end of six hours its average hydrogen-ion concentration is pH 8.55. The increase in alkalinity is probably due to the evolution of carbon dioxide and the consequent formation of sodium carbonate from sodium bicarbonate in the fluid. Our average of a number of determinations at the end of twenty-four hours was pH 8.85.

Considering, then, the alkalinity of the spinal fluid, and the hydrogen-ion concentration of the colloidal solution, one would expect in the tests as set up a curve in which the pH would gradually fall with the increasing dilution of the spinal fluid, approaching the hydrogen-ion concentration of

the colloidal solution as a base line.* It would be expected that in the colloidal benzoin test the curve would be more pronounced owing to the larger amounts of cerebrospinal fluid used and the lower pH value of colloidal benzoin solution. With colloidal gold, using a solution of higher pH value and much less spinal fluid, the curve would not be so marked. This is shown graphically in Chart 1. The figures given are the average of a number of determinations. The curve is fixed when the three factors (hydrogen-ion concentration of the colloidal solution, alkalinity of the spinal fluid and its dilution) are constant. It is the same with either negative or positive spinal fluids.

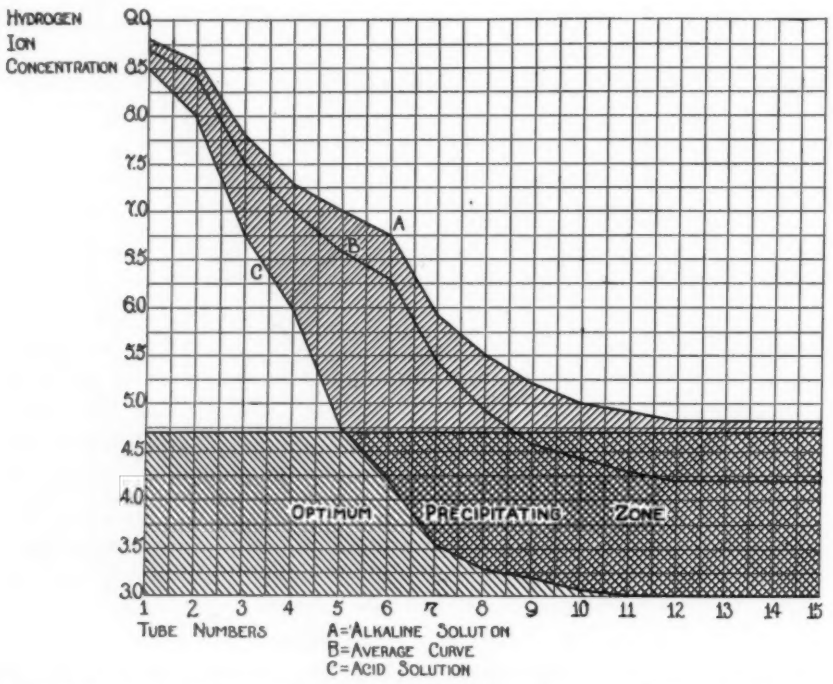

Chart 2.—Effect of variation in the hydrogen-ion concentration of colloidal benzoin on precipitation in Zones II and III.

Effect on precipitation of a change in hydrogen-ion concentration of the colloidal solutions.—Very slight changes in hydrogen-ion concentration suffice to affect materially the type of the curve of precipitation in a spinal fluid. This is shown in the tabulation.

From a study of the tabulation it is evident that the hydrogen-ion concentration of the colloidal solution plays an important part in determining the type of precipitation curve. These observations suggest that some of the technical difficulties with these reactions may be due to such slight variations.

Brom-cresol-purple and brom-thymol-blue seem to offer a better contrast than alizarin for the end-point determination in titrating gold sol.

*It is unfortunate and confusing that the pH varies as the logarithm of hydrogen-ion concentration. In order to avoid this confusion changes in hydrogen-ion concentration are uniformly spoken of in terms of pH.

A finer gradation than that afforded by the conventional alizarin method, should be used. In this study 5 drops of the indicator were added to 10 c.c. of colloidal gold, and $\frac{N}{100}$ acid or alkali added with 0.1 c.c. pipette to the first tinge of purple with brom-cresol-purple (pH 6.2 to 6.4), and to the first tinge of blue with brom-thymol-blue (pH 6.6 to 6.8). An amount of acid or alkali half way between the two calculations was used instead of making an attempt to gauge a range of pH 6.4 to 6.6 with brom-cresol-purple alone.

Cleanliness of glassware, age of spinal fluid, age of colloidal solution and so forth are other causes of variation in hydrogen-ion concentration.

If variations in the hydrogen-ion concentration of colloidal solutions affect precipitation it should be expected that the precipitation would be influenced by reducing the alkalinity of the cerebrospinal fluid. Such a procedure would lower the curve of hydrogen-ion concentration in the individual tubes. The following experiments were carried out to test this supposition.

Effect of reducing the pH value of the cerebrospinal fluid.—Spinal fluid is very highly buffered against variations in acidity or alkalinity, and it takes a surprising amount of $\frac{N}{50}$ hydrochloric acid to break this protection. Colloidal gold tests performed on spinal fluid after reduction to pH 6.4 gave precipitations such as

Original reaction	0123321000
With spinal fluid pH 6.4	5543321100

Similar tests with colloidal benzoin after reduction of the spinal fluid to pH 4.6 gave precipitations such as

Original reaction	000,000,022,000,000
With spinal fluid pH 4.6	333,333,333,000,000

Inasmuch as the hydrogen-ion concentration of the spinal fluid in these reactions is the same as that of the colloidal solution being used in the tests, the hydrogen-ion concentration in all tubes will be alike, and will fall within the optimal precipitation zone. The amount of precipitation is then merely a question of dilution and of the amount of precipitating substance present in the spinal fluid.

Adjusting the reaction to the optimal precipitating point in the individual tubes.—The results obtained by reducing the pH value of the cerebrospinal fluid to that more suitable for precipitation, suggest that similar results should accompany reduction of the pH value in the individual tubes immediately after setting up the tests. Those tubes of the following colloidal benzoin test which showed a pH value above 4.6 were accordingly adjusted to a uniform hydrogen-ion concentration of pH 4.6 by addition of $\frac{N}{50}$ hydrochloric acid.

Original fluid	000,000,033,300,000
Individual tubes adjusted to a maximum of pH 4.6	333,333,330,000,000

It is apparent that the reaction parallels that obtained by reduction of the pH value in the cerebrospinal fluid.

These experiments seem to show clearly the importance of the hydrogen-ion concentration in the individual tubes in determining the type of precipitation that occurs. They do not, of course, explain the nature of the precipitating substance. It is believed that the precipitating substance causing Zone II and III reactions is present in normal spinal fluids, but usually not in sufficient quantity to cause precipitation with colloidal solutions to any marked extent. When the inhibitory effect of the higher pH of the tubes to the left of these zones is removed, as in the last experiments, precipitation occurs in Zone I even in normal fluids. The precipitating agent, which for convenience is called substance "A" is pathologically increased when mesodermal or meningeal involvement is present. It seemingly produces most complete precipitation at a pH of 4.8 or below in the case of colloidal benzoin, and pH 6.9 or below with colloidal gold. Changes in the hydrogen-ion concentration of the colloidal solution, as shown for colloidal benzoin in Chart 2, very materially influence the zone of precipitation. Although substance "A" is present in increasingly larger amounts in the first tubes of the series, the increasingly higher pH value in these tubes prevents its precipitating action on the colloid. When pathologically increased, the precipitating substance "A" is present in sufficient amounts in the tubes to the right, which have an optimal hydrogen-ion concentration, to cause precipitation.

When destructive ectodermal or parenchymatous changes are present as in paresis, precipitation occurs in Zone I in spite of a high pH value in these tubes. This must be owing to the presence of another precipitating agent having a higher optimal precipitation point, which for convenience is called substance "B".* It probably occurs in much smaller amounts. This is shown by the fact that it only causes precipitation in Zone I or in those tubes having greater amounts of spinal fluid.

Precipitation of spinal fluid by ammonium sulphate.—Weston, Cruickshank, and Mellanby and Davies have shown that the active precipitating agent in cerebrospinal fluid may be itself completely precipitated by half saturated ammonium sulphate. This precipitate redissolved and the solution made up to the original quantity of spinal fluid, shows an augmented effect in precipitating colloidal benzoin or colloidal gold. I have been able to confirm this observation. The augmented effect is apparent not only in fluids from paretic, syphilitic and meningeal cases, but also in normal fluids. The effect can be further augmented (precipitation extended to the right)

*Since the completion of this study two excellent articles by Wright and Kermack have been published on "The properties of colloidal gum benzoin" and "The mechanism of precipitation of colloidal gum benzoin by cerebrospinal fluid." These authors are, therefore, entitled to priority for this statement. Attacking the subject from a very scientific chemical point of view in relation to the effect of hydrogen-ion concentration on the precipitation of colloidal benzoin they reach a conclusion with which mine is identical. The work here reported follows similar but simpler lines, and my conclusions were reached independently.

by making up the solution of the precipitate more concentrated than in the original spinal fluid.

COLLOIDAL GOLD

Original fluid	0122100000
Solution of ammonium sulphate precipitate	5554320000

COLLOIDAL BENZOIN

Original fluid	000,000,033,200,000
Solution of ammonium sulphate precipitate	233,333,330,000,000

Mellanby and Anwyl-Davies assert that this augmented effect suggests that spinal fluid contains a substance which inhibits the precipitation of colloidal solutions and that this inhibitory substance is not completely precipitated by half saturated ammonium sulphate. These authors leave the impression that this inhibitory action may be due to albumin or "pseudoglobulin." It is readily seen, from the results of the present study, that there is a close similarity between this type of reaction and that which occurs on reducing the pH of the spinal fluid. I contend that this inhibitory influence is exerted, not by albumin or pseudoglobulin, but by the normal alkalinity of the cerebrospinal fluid. Half saturated ammonium sulphate may precipitate the precipitating agent and especially substance "A" but the washed and redissolved precipitate with which various investigators have worked, no longer possesses the alkalinity of the original spinal fluid and its buffer salts. In support of this contention, the ammonium sulphate precipitate of spinal fluid was dissolved in a buffered solution of boric acid, potassium chloride and sodium hydroxide, (boric acid, potassium chloride—sodium hydroxide mixture) having a hydrogen-ion concentration of pH 8.4. The resultant reactions with colloidal benzoin were as follows:

Original fluid	000,000,220,000,000
Solution of ammonium sulphate precipitate in water	333,333,300,000,000
Solution of ammonium sulphate precipitate in buffered solution pH 8.4	000,003,210,000,000

TABLE I

pH	COLLOIDAL BENZOIN	pH	COLLOIDAL GOLD
	NORMAL FLUIDS		
3.4	000,000,033,333,000	6.0	0234554200
4.2*	000,000,011,000,000	6.3	0124432000
4.6	000,000,000,000,000	6.6*	0001110000
		6.8	0000000000
	FLUIDS FROM SYPHILITIC CASES		
3.4	002,012,333,333,000	6.0	2455555520
4.2*	001,000,333,320,000	6.3	1255543200
4.6	000,000,333,000,000	6.6*	0135542000
		6.8	0013310000
	FLUIDS FROM PARETIC CASES		
3.4	233,333,333,333,200	6.0	5555555542
4.2*	023,333,333,333,200	6.3	5555555420
4.6	023,320,033,331,000	6.6*	5555433200
5.0	023,310,000,000,000	6.8	5554331000
6.0	003,000,000,000,000		

*Proper pH

It may be seen that the last curve closely resembles the first. This suggests that the hydrogen-ion concentration probably plays the important part. To rule out any possible inhibitory effect from the buffer solution, the test was set up with the ammonium sulphate precipitate in distilled water and the hydrogen-ion concentration in the individual tubes immediately adjusted to approximately that shown in Chart 1 with the result as recorded.

In all these reactions it is conceded that the addition of alkali or acid of itself may have some effect on precipitation besides that directly due to the change in hydrogen-ion concentration. It is hoped that this factor can be made the subject of a future study.

CONCLUSIONS

1. The hydrogen-ion concentration plays a highly important part in determining the types of precipitation produced by spinal fluid and colloidal benzoin or colloidal gold solutions.

2. The hydrogen-ion concentration in the individual tubes in either reaction gives a curve from pH 8.7 to 4.2 for colloidal benzoin and from pH 7.4 to 6.4 for colloidal gold. This variation in the individual tubes determines the zones of precipitation.

3. The factors affecting this curve are: the alkalinity of the spinal fluid, the dilution of the spinal fluid, and the hydrogen-ion concentration of the colloidal solution.

4. There are seemingly two substances in spinal fluid causing precipitation of colloidal solutions. Substance "A" causes precipitation in Zones II and III, and only acts as a precipitant when the pH is below 4.8 for colloidal benzoin solution, and 6.9 for colloidal gold solution. It is the substance most affected by variations in hydrogen-ion concentration, as determined by the above factors.

Substance "B" causes precipitation in Zone I, and is present especially in paretic fluid. It has a much higher optimal precipitating pH value and is less affected than substance "A" by variation in hydrogen-ion concentration.

5. The augmented precipitating effect produced by precipitating the active substances from spinal fluid by half saturated ammonium sulphate is probably due to the removal of the inhibitory influence on precipitation of the normal alkalinity of the cerebrospinal fluid, rather than the removal of substances inhibiting precipitation, such as albumin or pseudoglobulin.

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PERSONAL EXPERIENCES WITH THE CULTIVATION OF TUBERCLE BACILLI AND THE USE OF THE GUINEA PIG AS A DIAGNOSTIC TEST ANIMAL FOR TUBERCULOSIS*

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THE clinical pathologist is often confronted by the internist and surgeon with ideas on the cultivation and guinea-pig inoculation of tubercle bacilli for diagnostic purposes which have been gleaned from sporadic perusal of brief articles in the scientific literature based upon insufficient evidence which has not been borne out by repeated, more elaborate, and controlled studies. These ideas, however, seem to have created a strong impression upon certain of our impatient medical colleagues who wish to believe that the laws of nature can always be manipulated at their will.

For the past twelve years the author and his coworkers have been cultivating tubercle bacilli and been looking for methods to hasten the growth of the bacilli upon artificial mediums, besides studying the development of tuberculosis in guinea pigs consequent to graded infecting doses and after the animals had been manipulated in different ways. It is for the purpose of crystallizing these observations and presenting the views derived therefrom to the clinical pathologist for his ready reference that this paper is submitted.

THE CULTIVATION OF LABORATORY STRAINS OF TUBERCLE BACILLI

The laboratory investigator of tuberculosis until quite recently was accustomed to perpetuating several strains of human and bovine tubercle bacilli, about which he had a great deal of intimate knowledge gained as a result of years of association. These strains usually grew well on the ordinary laboratory mediums such as five per cent glycerol agar, Dorset's egg medium and inspissated serum mediums. It is well known that both human and bovine tubercle bacilli are facultative aerobes and for this reason growth in liquid mediums, such as five per cent glycerol broth or any of the synthetic broths containing the necessary requisites for bacterial growth including glycerol, are usually confined to the surface of the liquid medium while in the deeper layers or sediment no appreciable growth occurs.

Aside from containing certain basic essentials for the growth of the lower forms of life, in general a great many mediums are suitable for the growth of the tubercle bacillus, and old laboratory strains, especially, grow about as well on one of these mediums as upon the other. The mediums used as

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a routine for the cultivation of old laboratory strains of human and bovine tubercle bacilli in the Research Laboratories of the National Jewish Hospital for Consumptives are five per cent glycerol agar and five per cent glycerol broth, not because they are better than other mediums but because they serve just as well. Laboratory strains of bovine tubercle bacilli that have been kept under artificial cultivation for some time grow about equally well upon glycerol containing mediums as upon those free from it. For the perpetuation of the strains only, glycerol agar in small bacteriologic test tubes is the more desirable medium, while glycerol broth is to be preferred when a large amount of an individual strain is desired, the surface growth being shaken down at intervals when profuse, to permit of additional development until the medium is exhausted, or becomes inhibitory to the further growth of the bacillus. The reaction of the medium need only be crudely tested by means of litmus paper, since laboratory strains of the tubercle bacillus will grow readily and about equally well within wide ranges of acidity, pH 6 to 8.

Regarding the incubation, it is almost needless to repeat, since this is common knowledge, that the incubator should be kept absolutely dark or the culture tubes be placed in light proof receptables, and preferably be kept at a uniform temperature of about 37° C., although ranges from 36 to 38° C. produce about equal results insofar as the growth of laboratory strains are concerned. That there is a difference in the growth ability of individual laboratory strains upon the same medium is readily noted by anyone who has grown several cultures through a number of transplants. The factors concerned in this growth difference existing between different cultures has not been accurately determined but seems to be inherent in and dependent upon, the individual strain. There is, however, a factor which has been repeatedly observed as important in the determination of a good growth regardless of the strain dealt with, whether human or bovine, or the medium used, provided however, the latter is one of the standards containing all the necessary requisites for growth of the tubercle bacilli. If the transplants are seeded from very fine suspensions, or too lightly, the majority will not develop, while with fairly uniform seeding of suspensions that are not too fine, the yield from a laboratory strain may be as high as 100 per cent excluding possible contaminations.[1, 2]

Another factor which has been found to be extremely important, especially to retain active growth and virulence in laboratory strains, has been the age of the parent culture from which the transplant is being made. In this respect human and bovine bacilli differ slightly[3]; the bovine bacillus being more resistant than the human bacillus. Fully grown cultures of human tubercle bacilli may remain viable on Petroff's gentian violet egg medium or five per cent glycerol agar, whether kept in the ice box or in the incubator, for from about four to eight months, so that successful transplants can be obtained on these mediums up to this time. Bovine tubercle bacilli may remain viable (in the sense of furnishing subcultures) for from about eight to sixteen months (the latter in exceptional cases). It is noteworthy that

guinea pig inoculation is still successful after subculturing has failed[4]. Drying of the medium tends to shorten the period of viability; also the more vigorous and profuse growers seem to give successful transplants after longer intervals, dated from the time of seeding, than the less vigorous.

THE ISOLATION OF TUBERCLE BACILLI BY DIRECT CULTURE METHODS

Of the various methods suggested for the cultivation of tubercle bacilli from contaminated tuberculous materials there are two that have survived the usual trials of persistent laboratory tests, the original and modified Griffith antiformin method, and the Petroff sodium hydroxide method. The latter method has suffered practically no important modifications since its first introduction in 1915 while the technic in the former has been altered a number of times by the originator since its introduction in 1914. There are many points of technic in which the sodium hydroxide method surpasses the antiformin method, and comparative exhaustive studies of the details of the two methods in the laboratories of the Research Department of the National Jewish Hospital for Consumptives resulted in drawing the following conclusions concerning these methods[5]: A comparison of the antiformin (Griffith's) and sodium hydroxide (Petroff's) methods shows the sodium hydroxide to be decidedly superior to the antiformin method. The advantages of time-saving and ease of manipulation, possessed by the simplified antiformin method, are gained at the expense of a decided decrease in the percentage of positive cultural results for human tubercle bacilli. If the sedimentation procedure is resorted to in both cases, which would seem highly advisable, the sodium hydroxide (Petroff's) method possesses the advantages of working with a more stable, more easily obtainable, less irritating and more economical reagent. While thus the sodium hydroxide procedure of Petroff's method is to be preferred, likewise the addition of the gentian violet (1:10,000) to the egg medium recommended by him, is also an advance over the Dorset's egg medium in that the gentian violet has no appreciable influence upon the growth of the tubercle bacillus on the egg medium but prevents the growth of certain contaminators that may have survived the sodium hydroxide treatment[6]. Although our experience leads us to believe that the Petroff's method for the isolation of tubercle bacilli from contaminated sources is by far the best method thus far described in the literature, our own results have never yielded the high per cent positive culture results recorded in the early reports with this method. Prolonged experience with this method has not changed the original figures of about thirty per cent yield on primary cultures from microscopically positive sputums[7]. There are many factors involved in obtaining positive cultures with tubercle bacilli and, in consideration of these, this low figure is easily explicable not as an index of poor technic but rather as a result of biologic factors which require perfect conditions, hardly obtainable where the bacilli are scattered in tissue debris, and where highly parasitic bacilli are to be adapted to a saprophytic life on culture mediums, not to mention the possibility that many of the bacilli in the tissues may be nonviable, a point remaining for future researches to elucidate.

THE DIFFERENCE IN GROWTH ABILITY OF DIFFERENT STRAINS OF "LABORATORY" AND RECENTLY ISOLATED CULTURES OF TUBERCLE BACILLI

In partial explanation of the fact that the percentage successes upon primary isolation of tubercle bacilli from contaminated materials does not approach the 100 per cent mark is the fact that there is a decided difference in growth ability upon artificial mediums of recently isolated and "Laboratory" strains of human tubercle bacilli. Just as laboratory strains gradually tend to lose their virulence under prolonged artificial cultivation so also do they tend to give more regular and profuse growth upon artificial mediums. The latter is, however, not to be inferred as meaning that all recently isolated cultures grow poorly upon artificial mediums or that all "laboratory" strains tend to become profuse growers with prolonged artificial cultivation. Some of our cultures even on primary isolation gave vigorous and profuse growth while others even after years of artificial cultivation grow scantily on mostly all artificial mediums. A cultural study with thirty-nine strains of human tubercle bacilli, twenty-eight isolated shortly before this study was initiated, upon 16 different mediums containing milk, egg, blood and various tissues, revealed that there is a distinct difference in the growth ability of different strains of human tubercle bacilli freshly isolated from the sputum and those artificially cultivated for long periods. Those recently isolated from the sputum, grow more sparsely on these mediums than do those artificially cultivated for long periods, and growth is obtained only on a few of the mediums, especially those containing inspissated egg and blood. Some of the recently isolated cultures fail to grow on all the mediums used. Human tubercle bacilli artificially cultivated for about one year grow well upon mediums containing besides agar, ammonium phosphate, salt and glycerol, any of the following: beef extract and peptone, defibrinated rabbit's blood, egg and ground up tissues (testes, liver, brain) sterilized by inspissation. Cow's milk or sodium caseinate has no appreciable effect upon growth, while sodium nucleinate and yeast nuclein have an effect, though this is not as marked as that seen with blood or egg.[8] Aminoids added to mediums have either an inhibitory influence upon growth or none at all.[9] If then we are requested to choose a suitable single medium for the cultivation of recently isolated human or bovine tubercle bacilli we should prefer an inspissated egg medium either as Petroff's gentian violet egg medium or Dorset's egg medium, with or without glycerol, as the case may demand for either human or bovine bacilli. Of equal value are the inspissated serum, blood or liver tissue mediums, but rather more difficult to obtain in large quantities fresh for laboratory use. It must be remembered that tissues which are not fresh may develop tuberculo-toxic tissue autolysates which may act in an inhibitory manner in mediums.[10] It is for this reason that tubercle bacilli cannot be grown upon fresh tissues or upon substances which undergo autolytic changes. The autolytic changes are especially enhanced by prolonged incubation as is required to grow tubercle bacilli.

After the bacilli have been acclimated to artificial cultivation for about a year, or earlier in the case of certain profuse growers, they can be trans-

ferred to glycerol agar or in special cases to glycerol broth, and this applies to both human and bovine bacilli, upon which they may be perpetuated indefinitely, being transplanted at monthly or bimonthly intervals.

THE GUINEA-PIG DIAGNOSIS FOR TUBERCULOSIS. NATURAL COURSE OF INFECTION

The guinea pig is par excellence the animal of choice for the inoculation diagnosis or testing for tuberculosis. This is an undisputed, universally accepted fact. The progress of the disease in this animal following the injection of human or bovine bacilli is much more rapid for equal dose than in any other economical and easily obtainable laboratory test animal. Aside from this the guinea pig is easily taken care of and handled. Regarding the mode of injection of suspected tuberculous material there is, however, no such unanimity of opinion. The most common, earliest described and usual modes of injection were the intraperitoneal (the weight of the animal being used as an index of the progress of disease), and the subcutaneous routes, the latter being used by Salus (1903) who paid attention to the local gland enlargement. At times combined intraperitoneal and subcutaneous injections have been resorted to. The intraocular inoculation of suspected material has been advocated by Baumgarten and later by Damsch but technical difficulties have placed it in disfavor as a routine test method. Nattan-Larrier and Griffon (1903) injected the suspected material into the lactating mammary gland of the guinea pig, and report favorable results from examination of the mammary secretions within eight to fifteen days, although controls were lacking. The lack of an ample supply of suitable animals is a common criticism of this method. In an effort to improve on these methods Bloch (1907) utilized the prevalent conception of the times, that bacteria developed more rapidly in injured tissues, and crushed the regional gland after subcutaneous injection of the suspected material. Smegma bacilli completely disappeared from the local gland within nine days while tubercle bacilli were numerous and evident after this period. The advantage of this method lay in the fact that examination of the regional gland did not necessitate sacrificing the guinea pig and subsequent autopsy could corroborate the preliminary diagnosis.

In the belief that the liver becomes involved with tuberculosis early in the guinea pig, Oppenheimer (1911) injected the suspected material into this organ at several places and claims to have obtained a miliary tuberculosis of the liver and spleen within five days when numerous and virulent bacilli were present in the original material injected, the usual time required being, however, sixteen days. He evidently was not aware, however, that the liver is one of the most resistant organs in the body to tuberculosis infection.[11]

Esch (1913) utilizing the Roemer intracutaneous tuberculin test for determining the presence of tuberculosis in guinea pigs compared the intraperitoneal, the gland-crushing method of Bloch, and the intrahepatic method of Oppenheimer and found that the latter method of inoculation had no advantage over the former two, and that of these the intraperitoneal inoculation had no advantage over the Bloch method. He found that a further

acceleration could be obtained only by introducing the tubercle bacilli into the blood stream by intracardiac or intravenous injection; a method only serviceable when a clear fluid is available. Autopsy is performed when the animal reacts positively to the intracutaneous tuberculin test.[12]

Recently Schulz (1923)[13] after reviewing some of the common methods in vogue prefers the intracutaneous injection of the suspected material into the guinea pigs. He admits, however, that local abscess formation with discharge of the material is a drawback to this method, the usual interval of waiting for systemic disease in the animal then being necessary.

It is rather interesting that only years of experience with inoculations of tubercle bacilli in graded and known amounts into guinea pigs convinces one that such injections into various parts of the body can lead to only slight and relative, but not practical, differences in the rate of development of the tuberculosis at the local site of injection, or in the development of the systemic disease as the case may be. When one is conversant with these facts the practical differences are in most cases too slight to be of great importance for diagnostic haste and the choice resolves itself into a consideration of the material to be injected and the individual preference of the clinical pathologist. For this reason it may not be amiss to briefly review some of our personal experiences with graded inoculations of tubercle bacilli into guinea pigs and those of some few other workers in this field whose experience is not limited, to an occasional laboratory diagnostic test with uncertain material, but covers a period of years and with large series of well controlled animals.

Krause has shown that following the subcutaneous injection of tubercle bacilli there is a rapid early general dissemination of the bacilli along the lymphatic channels in guinea pigs, which was corroborated in our laboratory and elaborated upon by using graded amounts of tubercle bacilli.[14] A total of 279 animals are tabulated in the latter study, the entire indicating that the macroscopic tuberculous involvement of the guinea pigs follows a definite organ sequence which is related to the time interval and can be correlated fairly well with the dose of bacilli used for infecting. It is also pointed out that studies of this type should be carried out on fairly large series of animals on account of the possibility for error when only a few animals are used. There is, therefore, a definite growth power of the bacilli in the guinea pig following subcutaneous inoculation which is dependent upon the individual organism (virulence), the dosage and the time element, just as occurs in the test tube where, however, the virulence factor does not come into play. Caution is, therefore, also necessary in utilizing for drawing scientific conclusions, any material such as pus, sputum or urine where none except the time factor can be known. Our own experience with intracutaneous, intraperitoneal, and intravenous injections of graded doses of tubercle bacilli bear out those cited above for the subcutaneous injection with, however, these slight modifications, especially considering the most striking difference in the case of the intravenous and the subcutaneous administrations of the same amounts and same culture of bacilli. In case

the bacilli have been injected subcutaneously the local, nearest tributary, glands begin to develop early (dependent upon the dose within about two weeks to one to two months, the latter with very small doses of virulent bacilli) and at a rate which slightly exceeds the development of the disease in the spleen, lungs, and various glands, especially those tributary to the lungs following the same dose given intravenously. While the local tributary glands following subcutaneous inoculation exceed slightly the organ development in the intravenous inoculations, the organ involvement of the spleen, lungs and tributary internal glands, especially the peribronchials and peritracheals, following subcutaneous inoculation lag slightly behind those in the intravenously injected animals. When the inoculations are given intraperitoneally the abdominal organs, especially the spleen, and glandular involvement occurs about coincidently with the local gland involvement by the subcutaneous method while the general involvement occurs earlier by the intraperitoneal method. Following intracutaneous inoculation of the same amounts of bacilli as by subcutaneous inoculation there is very little practical difference in the rate of development in the disease from the latter, both in local gland or general development of the disease. Although it must be admitted in the final analysis of the choice that when comparing these various methods one is confronted with the determination as to the desires at hand. If a general disease is demanded the intraperitoneal, intrahepatic or intravenous methods of injection must be the choice, since the internal organs are affected earlier and more efficiently than following either intracutaneous or subcutaneous infections with the same dose of bacilli. If it is, however, a question of earlier diagnosis, and, the finding of tubercle bacilli with definite local glandular enlargement is accepted as a criterion following intracutaneous or subcutaneous infections, and, organ or internal glandular involvement following intraperitoneal, intrahepatic, intracardiac or intravenous injection, it must be admitted that there is very little practical difference between these methods. There is, however, a consideration which merits attention in comparing these methods and that is that for a determination to be made by the intraperitoneal, intrahepatic, intracardiac and intravenous methods the animals must be sacrificed, while by the intracutaneous and subcutaneous methods the animals can be submitted to repeated examinations of the local glands, provided, of course, contamination from external sources is avoided to prevent erroneous diagnoses and finally the diagnosis can then be verified at postmortem after several months. Of the intracutaneous and subcutaneous methods the former has the disadvantage that the local lesions very frequently rupture and are then a source for contamination and wrong diagnoses. The tendency for such errors is far less and not at all common by the subcutaneous method of inoculation and in the latter the disease in no wise develops more slowly than by the intracutaneous method. Tuberculin or other tests, which may err at times, are not necessary when utilizing the intra- or subcutaneous methods since observation itself is ample. Gland crushing to hasten the local disease does not seem to have gained much favor among tuberculosis workers and our own experience in well controlled

experiments revealed no differences betwen control guinea pigs and those in which the glands were crushed.[15]

In passing, attention might also be drawn to the observation that guinea pigs which have been infected usually reveal evidences of the disease within two or three months after the time of inoculation and it is extremely rare to see the disease start to develop in these animals from the inoculation after three months, so that it may be laid down as a general rule that practically all inoculation test animals may be sacrificed for examination after that time.

It must also be recognized that tuberculosis developing in the guinea pig has certain definite characteristics which occasionally, to the inexperienced eye, may be simulated by laboratory epidemics of pseudotuberculosis. Tuberculosis in the guinea pig is primarily a glandular disease and as such massive organ involvement, excepting the spleen, is the consequence of a massive generalized tuberculosis occurring late in the sequence of events, while pseudotuberculosis is primarily an organ disease and as such the liver is involved early while the glands are usually only slightly involved, if at all.[16, 17] Of course for practical purposes the finding of the tubercle bacillus, which should always be sought for to verify the macroscopic diagnosis, easily differentiates these two conditions, since acid-fast bacilli are not associated with pseudotuberculosis. The two diseases, pseudotuberculosis, occurring spontaneously, and tuberculosis by inoculation, may coexist. It is also noteworthy that in view of the high susceptibility of the guinea pig to inoculation tuberculosis, spontaneous disease is rarely encountered in spite of the fact that diseased and normal animals are commonly housed together in large numbers in experimental work. A good precaution with all diagnostic test animals, however, is to house them separately in small cages and thus obviate even the remote possibility of error, a precaution so easily taken. A word of caution is also necessary in the preservation of the suspected material to be used for inoculation. It should be kept, not longer than is absolutely necessary, in a light proof receptacle both during collection, if this requires any length of time as is the case with sputum, and preparation for inoculation. A concentration method is also to be recommended in many cases, especially when dealing with contaminated materials or those containing a large amount of debris; in our hands the sodium hydroxide method of Petroff has proved the most serviceable

EXPERIENCES WITH EXTRANEOUS AGENTS TO HASTEN TUBERCULOSIS IN THE GUINEA PIG

The mooted question which still merits careful consideration is the proper evaluation of the various factors in tuberculosis which constitute resistance; Can we attribute the major part to cellular action and, if so, what cells are to be incriminated, or are humoral factors weighty, and, if so, to what extent? In proposing such a division another consideration confronts us for the purpose at hand. Can the main factors concerned be sufficiently influenced by artificial means to alter the course of the disease, so that it will be important

on practical application? It has been the hope of many that such might be the case but evidently the complex which we call resistance in tuberculosis is either not confined to one specific definable element but rather is due to an interwoven complex of factors, interdependable. If it is confined to a single element, which is highly improbable, this is in an inapproachable condition or as a bare possibility still remains to be discovered.

The use of the leucotoxic agents such as the roentgen ray, benzene, thorium X and other radioactive substances, and applied heat is based upon an erroneous conception that the leucocytic changes seen in tuberculosis are cause rather than the effect of the changes coincident thereto. Exhaustive trials with the roentgen ray, benzene, thorium X,[18] mustard gas[19] and applied heat,[20] in large series of guinea pigs inoculated with graded doses of tubercle bacilli have yielded only negative results, even though many of the test animals developed an extreme leucopenia consequent to the treatment accorded them. These agents proved entirely without effect of any kind upon the progress of the tuberculosis in the guinea pigs and cannot, therefore, be recommended for practical application in hastening the guinea pig diagnosis for tuberculosis. It must not be assumed from what has been thus far stated that the spread and development of tuberculosis in the guinea pig cannot be influenced by any known method of experimentation, this would be rather too radical a conclusion and unjustified in view of even our own data and experiences. It would probably better answer the purpose to assert that we know no method which will hasten the development of tuberculosis in the guinea pig to be of practical value for giving earlier diagnostic information. Of the various local procedures tried it has been found that lamp black injected subcutaneously coincident with the tubercle bacilli has a distinctly retarding influence, while finely powdered glass had an appreciably accelerating influence, though not sufficient to be available for practical use in accelerating the guinea pig diagnosis for the presence of tubercle bacilli in pathologic fluids.[21] Likewise, cauterization of a local intracutaneous site of inoculation of virulent bacilli in guinea pigs when the dose is not excessive and when the local site is cauterized later than four days after infection, there results a distinctly greater amount of generalized tuberculosis as compared with control, untreated guinea pigs given only the intracutaneous inoculation of the bacilli. Cauterization of the local site of intracutaneous injection within four days has just the opposite effect and there is found distinctly less macroscopic tuberculosis in the guinea pigs.[22]

CONCLUSIONS

As a result of a long and extensive experience in cultivating human and bovine tubercle bacilli it is recommended that the preferred method for isolating these bacilli from tissues and contaminated materials is the Petroff's sodium hydroxide method combined with seeding on the gentian violet egg medium. The recently isolated bacilli grow about equally well upon egg, serum, blood and inspissated tissue mediums and choice is made of the egg medium only on the basis of its ready access in the laboratory. Human

bacilli do best when glycerol is added to these mediums while bovine bacilli prefer to grow without it. Laboratory strains of bacilli (after artificial cultivation of over a period of a year) grow equally well upon glycerol agar and are preferably continued on this medium or, under special circumstances, upon glycerol broth. Individual strains of bacilli reveal decided differences in growth ability upon all mediums, which seems to be an inherent characteristic of the strain concerned. No method is available at present which will hasten the growth of the bacilli appreciably upon these mediums, provided it is recognized that the seeding plays an important part in the subsequent growth of the bacilli. Tubercle bacilli lose their viability after prolonged residence in the incubator or ice box and transfers should be made at monthly intervals to perpetuate them.

The inoculation of guinea pigs for making the diagnosis of tuberculosis resolves itself into the question of the criterion to be used and whether or not haste is expedient. If the criterion is to be the finding of acid-fast bacilli and time is an item to be reckoned with, it would seem that the method of choice should be the subcutaneous injection of the suspected material properly prepared by preliminary sodium hyroxide treatment and neutralization, especially when it is from contaminated sources. As soon as local findings warrant, the glandular contents can be examined, using every precaution to avoid external tuberculous contamination which may vitiate the subsequent findings in the animal. The subcutaneous method of inoculation is preferred over the intracutaneous on account of the greater danger of ulceration and external contamination in the latter, even though it must be admitted that the disease develops at about the same rate in the guinea pig by both methods of inoculation. After local findings prove positive, the animal can be sacrificed at a later date when generalized disease has developed, to verify the diagnosis. Intravenous, intracardiac, intrahepatic and intraperitoneal inoculation results in rapid generalized disease in the guinea pig but this does not develop earlier than the local disease following the intracutaneous or subcutaneous inoculation of equal and graded doses of bacilli. The disadvantage of the latter methods of inoculation for diagnostic purposes is the necessity for complete examination of the entire animal at a time when there are no visible external signs of the disease. Recourse to methods, such as roentgen-ray exposure, injection of radioactive substances or other leucotoxic agents are based on erroneous observations and do not hasten the development of tuberculosis in the guinea pig. A few procedures such as the coincident injection of powdered glass and cauterizing at an appropriate time have a slight effect but are of no practical significance for diagnostic purposes.

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LABORATORY METHODS

A TECHNIC FOR DEMONSTRATING RETICULUM FIBERS IN ZENKER-FIXED PARAFFIN SECTIONS*

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IT is the habit in many laboratories, to fix necropsy material in Zenker's fluid as a matter of routine, only employing formalin fixation in addition if there is any indication that silver-impregnations, fat-stains, etc., are to be done. The excellent fixation, the fact that the tissues are mordanted with chrome salts (enabling one to employ aniline as well as vegetable dyes as stains) and the precision of the nuclear staining with Zenker fixation, all tend to influence the pathologist in favor of this solution for routine purposes, in preference to the simpler and possibly less artificial formalin methods. Routine fixation in two fluids, however, is apt to be irksome, and the filing away of the two jars, one of which may never be reopened, is cumbersome. On the other hand, it is very embarrassing to find, after completing an autopsy, that all one's material has been fixed in Zenker's fluid and no formalin material is available for certain stains, the indications for which were not evident at the time the postmortem was performed.

In such a case it becomes necessary to devise some method of adapting these stains to Zenker-fixed tissues. There are two objections to using such tissues for silver impregnations: (1) The silver forms a chromate with the chromium salts in the cytoplasm of the cells, as well as in the fibers, which increases the density of the section and lessens the contrast between the reticulum and the parenchyma. (2) There is apt to be a precipitation of silver chromate in the interstices of the tissue, and over its surface, which renders the picture muddy and untidy and therefore unfit for photographic purposes. Schmorl,[1] in the 1921 edition of his textbook on laboratory technic writes: "The methods of fixation which depend upon metallic salts (especially chromium salts) alone appear to be unsuited" for silver impregnation. Mallory and Wright[2] in their "Pathological Technique" merely specify that the fixative should be 4 per cent formaldehyde, condemning the other fixations by inference.

Zenker's fluid contains 2.5 per cent of potassium dichromate, 5 per cent to 8 per cent of mercuric chloride, and 5 per cent of acetic acid, in water. The sulphate of soda originally included in the formula, has been omitted ever since it was discovered that it serves no useful purpose. After using

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this fixative, then, we must remove the metallic salts of mercury and chromium. It is already part of our staining routine to remove the mercury with a weak alcoholic solution of iodine, getting rid of the chromium is scarcely more difficult. In the Pal modification of the Weigert hematoxylin stain for nerve tissue the chromium lakes, formed in combination with the hematoxylin, are partially or wholly removed during the process of differentiation, by means of potassium permanganate, which replaces and oxidizes the chromium salts and is then removed together with them by means of an oxalic or sulphurous acid solution

As potassium permanganate and oxalic acid solutions are in frequent use in the pathologic laboratory, usually in 0.25 per cent and 5 per cent concentrations respectively, they have been employed in the following method. The permanganate replaces and oxidizes any chromium compounds in the tissues and renders them soluble, the oxalic acid removes the permanganate and any chromium salts that may have remained unaffected by the manganese compound, and water removes the oxalic acid and soluble chromium salts. The method here given is in no way original, being merely a combination of two well-known technics, but it has the merit of making a satisfactory silver impregnation of Zenker-fixed sections possible.

Cut paraffin sections at 5μ-10μ, the thinner the better, and remove the paraffin in the usual manner, treating the slides with weak alcoholic iodine for five minutes to remove the mercury. Remove the iodine with a weak aqueous solution of thiosulphate of sodium (hypo), and wash in water. Immerse for five minutes in 0.25 per cent aqueous potassium permanganate, rinse in tap water, and treat for 15 to 30 minutes with a 5 per cent aqueous solution of oxalic acid, after which the slides are thoroughly washed in tap water to remove the now soluble chromates. A soaking in the oxalic acid bath overnight will accomplish the same results, provided that the sections are thoroughly washed out in the morning.

After rinsing in distilled water, the sections are next immersed in a solution of 2 per cent silver nitrate in distilled water for 48 hours, more or less. This is best done in subdued light. They are then washed thoroughly in distilled water and placed in a silver-ammonium oxide bath for half an hour. This is prepared fresh for each day's batch of slides, as it does not keep well. To 20 c.c. of a 10 per cent silver nitrate solution in distilled water, add 20 drops of a pure 40 per cent sodium hydroxide solution, also made up with distilled water. The resulting brownish precipitate is dissolved in strong ammonia water, which is added slowly, shaking continually. About 2 c.c. will be needed, so that it is well to add the ammonia drop by drop as this point is neared, until the precipitate is almost entirely dissolved. It is better to filter out a few undissolved grains than it is to run the risk of adding too much ammonia. The resulting solution is brought up to 80 c.c. by the addition of distilled water. After treating one box of slides the solution should always be refiltered before pouring it into another.

After a thorough rinsing with distilled water, the sections are brought into 5 per cent formalin (2 per cent formaldehyde solution) for another half hour, turning deep brown in this bath. After a rinsing in tap water

they are treated for an hour with 1 per cent aqueous gold chloride, in which the brown color fades and is replaced by a delicate violet gray. After washing in tap water the superfluous silver salts are removed by a two-minute bath in a 5 per cent aqueous thiosulphate of soda and then washed for several hours in tap water. The reticulum is now stained, but the nuclear impregnation is uneven, stippled and unsatisfactory and the sections lacking in detail. This is the Bielschowsky-Maresch method.

In order to remedy the defects just noted and to intensify the contrast between reticulum and collagen, the Weigert-Van Gieson stain is next superimposed upon the preceding. The slides are stained for one minute in Weigert's iron-hematoxylin, which is composed of two solutions: (1)

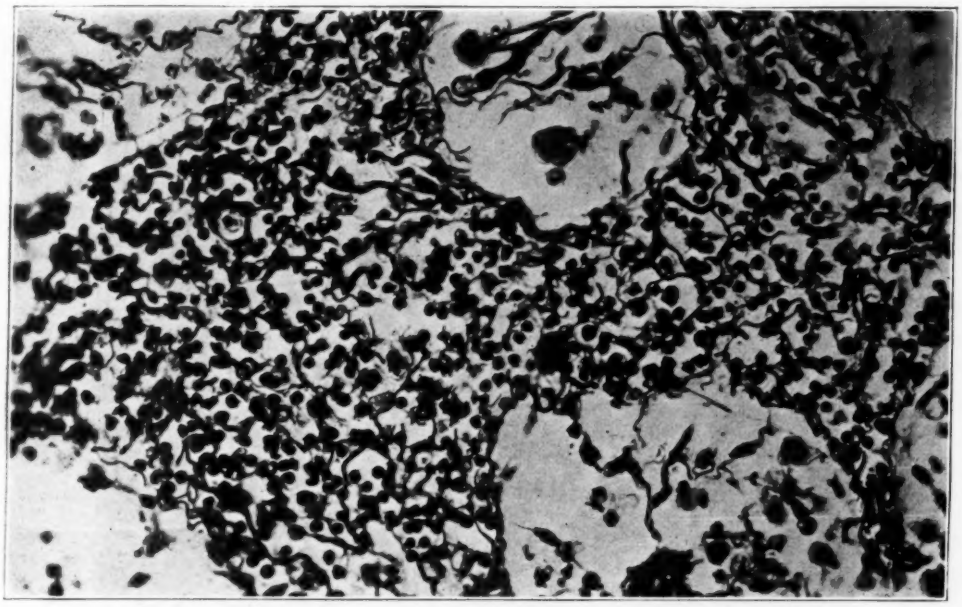

Fig. 1.—An edematous lymphnode from the mesentery, showing the lymphoid reticulum. x500.

one per cent ripened alcoholic hematoxylin, and (2) four parts of 40 per cent ferric chloride, one part hydrochloric acid, and ninety-five parts water. These are mixed in equal parts and a purplish-black ink should result; if it has a greenish or brownish color it is worthless and the indications are that solution 2 should be made up fresh, as it readily decomposes. The iron-hematoxylin stain should always be made up freshly for each day's work, as it does not keep. After staining in this lake the sections are washed until they are distinctly blue, after which they are counterstained in Van Gieson's picro-acid-fuchsin mixture for thirty seconds. (Ten c.c. aqueous 1 per cent acid fuchsin to 90 c.c. of aqueous picric acid solution saturated at room temperature.) Do not wash the sections in water, but run them rapidly through 95 per cent alcohol, absolute alcohol, and xylol, and mount in the usual manner in Canada balsam.

Note: The German Liquor Ferri Sesquichloratis called for in Weigert's original formula for iron-hematoxylin is replaced by the simpler 40 per cent ferric chloride solution, which appears to act equally well. The formula for the picro-acid-fuchsin stain given here is the one recommended by Weigert, and is superior to the original Van Gieson mixture.

The reticulum stain thus obtained is very fine and superior to those obtained by using either method alone. As shown in the accompanying photomicrographs (Figs. 1 and 2) the reticulum fibrils stand out sharply black, or dark violet. The collagen fibers exhibit varying shades of red or rose; the nuclei are black, bluish or brownish, depending upon how long they have been acted upon by water after the hematoxylin stain; the cytoplasm of the cells is grayish-yellow. Muscle fibers stain more brightly yel-

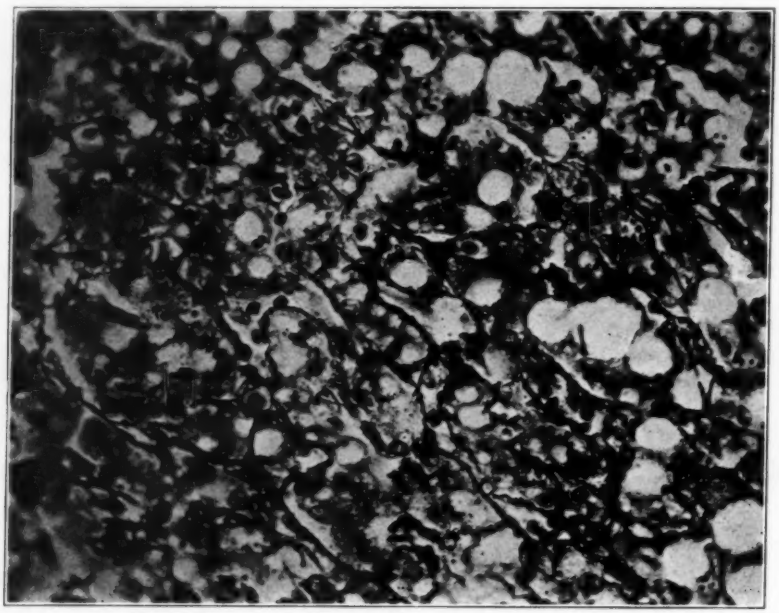

Fig. 2.—A section of liver with fatty infiltration, the reticulum is rather straight and coarse. The granules in the cytoplasm are pathologic, but not artifacts. x500.

low, as do elastic fibers. The very finest reticulum fibrils are brought out by this method, for if the silver does not penetrate them, the acid fuchsin will, so that they are sometimes beaded black on vermillion. Van Gieson's stain used alone is incapable of staining these fibers in this way, as proved by controls. The axis cylinders of nerves are also stained a deep black in this technic, which may prove well-suited to neuropathologic needs.

The method appears formidable when set forth in cold type, but in reality it is very easily performed and can be done readily whilst other things are being attended to, if one merely keeps track of the time that sections have been in a given bath by means of an interval timer of some sort. In the silver and gold baths a few minutes more or less make little difference, and the impregnation is remarkably uniform, in contrast with frozen-section experience. Set down in tabular form, the steps are as follows:

1. Remove paraffin from thin mounted sections in usual manner, and treat with weak alcoholic iodine and weak hypo, as in regular Zenker technic.
2. Five minutes in 0.25 per cent potassium permanganate, rinse.
3. 15 to 30 minutes in 5 per cent aqueous oxalic acid, wash thoroughly.
4. Rinse in distilled water and use distilled water for washing until after the sections have been treated with formalin.
5. 48 hours in 2 per cent aqueous silver nitrate, wash well.
6. One-half hour in silver-ammonium oxide solution, wash well.
7. One-half hour in 5 per cent formalin, rinse at tap.
8. One hour in 1 per cent gold chloride, rinse at tap.
9. Two minutes in 5 per cent hypo.
10. Wash thoroughly in tap water, several hours.
11. One minute in Weigert's iron hematoxylin.
12. Soak in tap water till blue.
13. Thirty seconds in Van Gieson's picro-acid-fuchsin.
14. Through alcohols and xylol and mount in balsam.

If these directions are followed with reasonable care, the method will be found to be perfectly satisfactory. Causes of trouble are: (1) Failure to prepare silver-ammonium oxide solution properly, or to filter it before use. (2) Failure to wash between the silver baths and the formalin bath (precipitates form). (3) Failure to use reasonably fresh iron chloride solution in preparing iron-hematoxylin, or to prepare the iron-hematoxylin lake fresh. The writer has had no experience in the lasting qualities of these preparations, but they stand strong sunlight well and it is believed that they will keep indefinitely.

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THE STABILITY OF KOLMER'S NEW ANTIGEN FOR COMPLEMENT-FIXATION TESTS IN SYPHILIS*

By Robert A. Kilduffe, B.A., M.A., M.D., Los Angeles, Calif.

As a result of the studies in the standardization of the Wassermann test conducted by himself and his associates Kolmer[1] has introduced a new antigen.

The work of numerous investigators, among whom may be noted Hartman and Reyner,[2] Schamberg and Greenbaum,[3] Lynch,[4] Rockwood and Sanford,[5] Kolmer and Denney,[6] Kilduffe,[7, 8, 9, 10] and others has shown this antigen to be remarkably delicate and reliable in complement-fixation tests for syphilis and possessed of a high degree of relative specificity, especially when used in the quantitative method of complement fixation described by

*Received for publication, February 22, 1924.

Kolmer[11] though these qualities are also evidenced when the extract is utilized in other methods.

As a result, the antigen is very widely used and, in all probability, will be still more widely utilized in the future, possessing as it does a remarkable range of antigenic activity coupled with a low anticomplementary value. Extracts anticomplementary in 0.5 c.c. of 1:4 or 1:6 and antigenic in 0.5 c.c. of 1:2400 can be almost uniformly prepared and antigenic units of 0.5 c.c. of 1:4000 are not uncommon, and I have even prepared one antigen by Kolmer's method which possessed the extraordinary antigenic unit of 0.5 c.c. of 1:8000.

As the final volume of the finished antigen is one hundred cubic centimeters, and as with a ten unit dose, an antigen having an antigenic titer of 1:2400 or 1:4000 suffices for a large number of tests, it becomes of practical interest to know something of the stability of the extract under the usual conditions obtaining. This is of interest to the inactive laboratory where only a small number of tests are run, and where one hundred centimeters of extract will, therefore, suffice for long periods, and also to the central laboratory supplying antigen to its branches as affecting the volume in which the antigen may be made and the frequency with which it must be prepared.

The present note is concerned with an observation on this point.

In 1922, while Director of Laboratories to the Pittsburgh Hospital, an antigen was prepared by Kolmer's method which, when titrated on April 6, 1922, had the following value:

Hemolytic: not in 0.5 c.c. of 1:4.

Anticomplementary: 0.5 c.c. of 1:4.

Antigenic: 0.5 c.c of 1:2400 (titration not carried higher).

This antigen was kept in a stoppered bottle in the refrigerator and sent by mail to Los Angeles in January, 1924. When retitrated January 25, 1924, it gave the following result:

Hemolytic: not in 0.5 c.c. of 1:4.

Anticomplementary: 0.5 c.c. of 1:6.

Antigenic: 0.5 c.c. of 1:2400 (titration not carried higher).

The only result of keeping the extract for twenty-one months, therefore, was a slight increase in the anticomplementary unit and it thus appears feasible to prepare antigens of this character in large volume and to store them, if desired, for long periods of time without deterioration of practical import, and without affecting their suitability for the conduct of complement-fixation tests for syphilis.

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A SIMPLIFIED METHOD FOR RECORDING SMOOTH MUSCLE MOVEMENTS*

BY GEORGE R. LOVE, M.D., CHICAGO, ILL.

THE graphic registration of intact smooth muscle while not a common student procedure is as simple as that of carotid blood pressure. Likewise, the effects of the autonomic and smooth muscle drugs are so distinct that the results are very instructive.

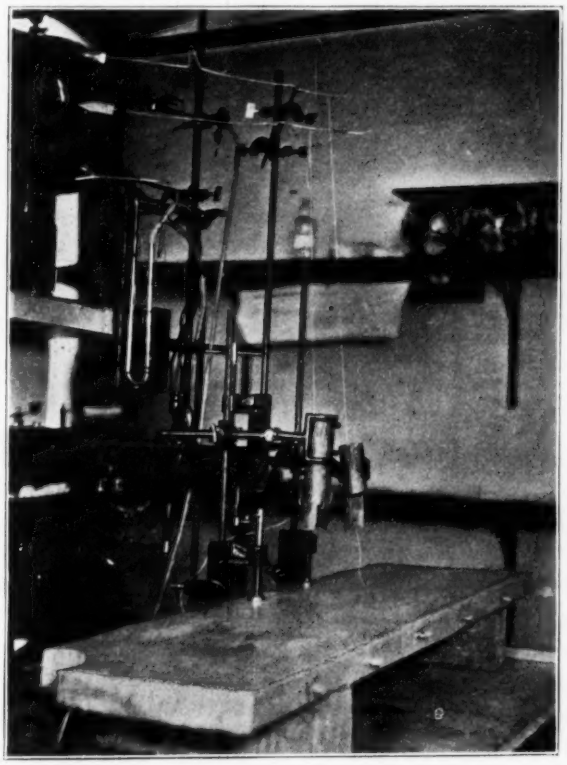

Fig. 1A:—The lead pipe on the left is cut for the intestines or uterus. The tissues may be anchored either in one or in two points. The pipe on the right is for the bladder with one anchor. The heavy clamps prevent respiratory fluctuations.

*Contribution from the Department of Pharmacology and Therapeutics, University of Illinois, College of Medicine.
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The method presented is merely an adjustment of the methods of Trendelenburg[1, 2] and Jackson.[3]

Lead pipes, 3 to 6 inches long and 1 to 1¼ inches in diameter, are cut on one end with a hack saw and bent in a shape to fit over the part to be tested. The rough edges are filed smooth, and after warming, the pipes are ready for use. The muscle may be anchored at two points and the lever attached between, or it may be anchored at one point and a free portion connected to the writing lever. (See Fig. 1.) The lead pipes are held in position by heavy clamps, thus abolishing all effects of respiration.

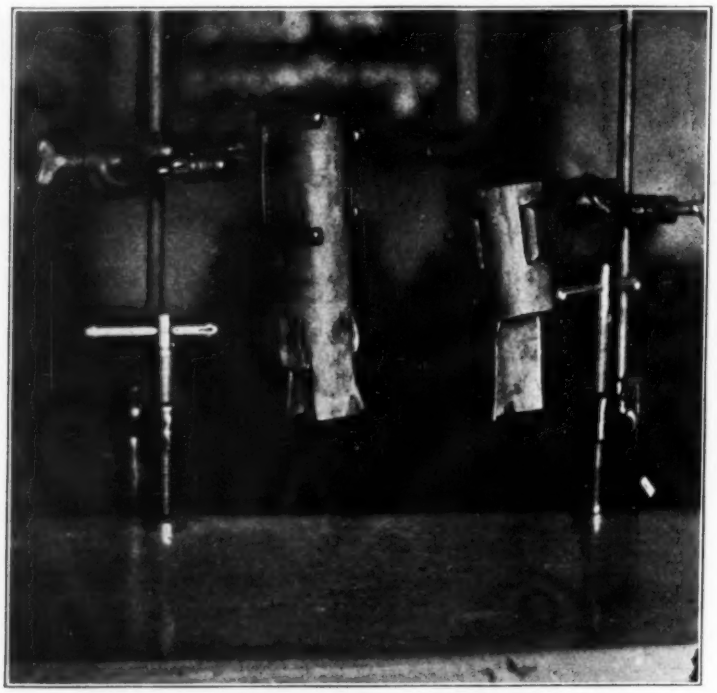

Fig. 1*B*.—An arrangement of the dog board to the kymograph, particularly adapted for class work on smooth muscle; pulleys are eliminated, the drum and recording levers are by position protected from many of the laboratory accidents.

A very convenient position of the apparatus is shown in the illustration. The mass, ease of preparation, and indestructible nature of the lead pipes make them better adapted for student work than glass tubes, since the transparency of glass possesses no particular advantage for the method. For light muscle, pipes of a smaller diameter may be used. We have used this method in the student laboratory for the past three years with very satisfactory results.

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AN AUTOMATIC SHORT-CIRCUITING DEVICE FOR INDUCTION SHOCKS*

By L. F. Shackell, M.D., Salt Lake City, Utah

IN carrying out certain experiments in the physiological laboratory, particularly in demonstrating fatigue in an excised muscle, the secondary coil of the inductorium must be pushed well over the primary coil so that the make shocks as well as break shocks may elicit maximal contractions of the muscle. Under these circumstances the complicating factor of excessively strong break shocks is introduced into the experiment. To obviate this factor in particular, the apparatus herein described was originally designed.

Figs. 1 and 2 show front and side views of the device as attached to the Harvard inductorium. Two metal posts, *a*, *a'*, are screwed into the vulcanite end of a Harvard inductorium, and connected by short wires to the secondary posts, *b*, *b'*. The latter are thus conveniently short-circuited through the flat spring *c*. This spring can be raised a trifle by the throw of the lever, *d*, which is actuated by the electromagnet, *e***, connected in the primary circuit of the inductorium. The amplitude of throw of the lever, *d*, may be altered somewhat by the set screw, *f* (Fig. 2).

Provision for raising and lowering the electromagnet *e*, through minute distances has been made as follows:

The short rod, *g*, which is screwed into the base of the electromagnet, slides in the cylinder, *h* (Fig. 2)—shown in vertical section in Fig. 1. To prevent twisting of the electromagnet and lever, a short pin, integral with the rod, *g*, slides in a vertical slot in the cylinder, *h*. Into the base of the rod, *g*, is screwed the upper end of the universal joint, *j*. This joint, with its attached screw, *k*, and the cylinder, *h*, have been adapted with but slight modification from the pressure regulator which obtained with the older style of Harvard capillary electrometer.

A suitable resistance to the turning of the adjusting screw, *k*, is provided by the short spiral spring, *m*, (Fig. 2).

It can be seen that the attachment above described does not interfere with the ordinary uses of the Harvard inductorium. All that is needed to isolate the attachment is to remove the spring, *c*, and leave the electromagnet, *e*, out of the primary circuit.

When the inductorium and attachment are arranged for single shocks, the attachment may be adjusted at will so that either make or break induction shocks are automatically short-circuited. If stimulation with break shocks only is desired, the electromagnet, *e*, is raised or lowered by means

*From the Physiological Laboratory, University of Utah, Salt Lake City.
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**See No. P1646, Baird and Tatlock catalog, 1922, ii, 250.

of the screw, *k*, until the throw of the lever, *d*, when the primary circuit is closed, is sufficient to raise the spring, *c*, about one-half millimeter above the post, *a*. If the set screw, *f*, is turned up as far as is consistent with positive action of the electromagnet, *e*, the lag in the throw of the lever, *d*, allows the make induction current, if not too strong, to shunt over the spring, *c*, before the connection between the posts *a* and *a'* is broken. When, on the other hand, the primary circuit is broken, the induced current is diverted through the tissue being stimulated by reason of the lag of the spring, *c*, in making contact with the post, *a*.

The automatic short-circuiting of break shocks is accomplished in the following manner: The screw, *k*, is turned until the lever, *d*, when held up, just clears the lower surface of the spring, *c*. One or two extra cells are connected into the primary circuit, so that the throw of the lever, *d*, occurs with a snap. The secondary coil is also pushed well in over the primary coil.

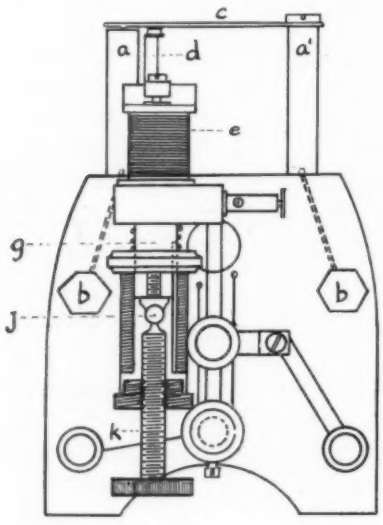

Fig. 1.

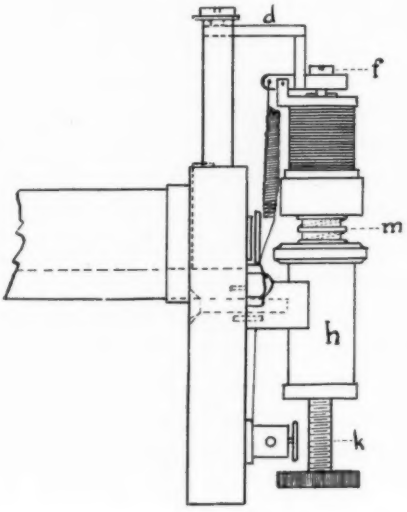

Fig. 2.

When the primary circuit is now closed, there is enough inertial overthrow of the lever, *d*, to raise the spring, *c*, for a minute fraction of a second, during which a residuum of the make induction current is thrown into the tissue. With the recoil of the lever, *d*, however, the spring, *c*, drops back against the post, *a*, thus providing a short circuit for the break shock when the primary circuit is opened.

In Fig. 3 are two tracings taken from a frog's gastrocnemius muscle, which was receiving 30 stimuli per minute. To indicate the character of the stimulation, a signal magnet was connected, along with the short-circuiting device, in the primary circuit of the inductorium. The down strokes of the signal magnet registered make shocks. The writing points of muscle lever and signal magnet were kept as nearly as possible in a vertical line.

In tracing A (Fig. 3) the make shocks, and in tracing B the break shocks were automatically short-circuited. Furthermore, in making tracing A, two

dry cells were used, and the secondary coil was set at 6 cm.; while in making tracing B four dry cells were used, and the secondary coil was set at 2 cm.

Among other uses to which the device has been put is the demonstration of graded submaximal stimulation of skeletal muscle. In this case short-

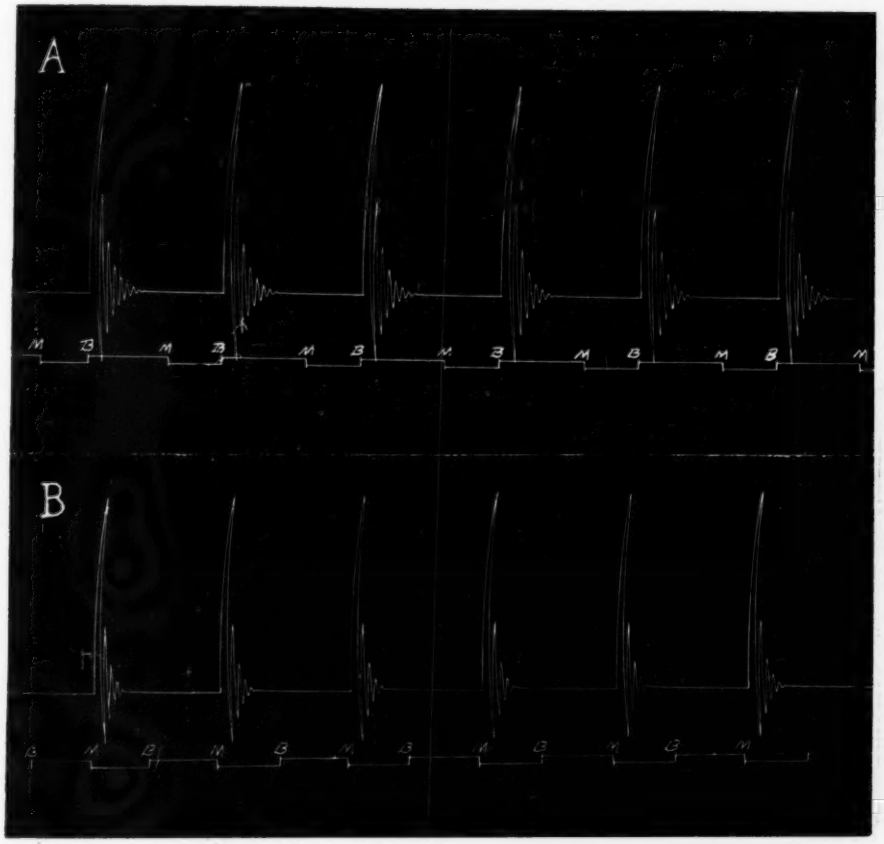

Fig. 3.

circuiting of the break shocks, with the secondary coil run in to about 3 cm., has seemed to give more consistent results than the use of break shocks with the secondary coil at some distance from the primary.

The writer is indebted to Mr. John Kohnhorst for valuable technical assistance.

APPARATUS FOR THE DETERMINATION OF THE BLOOD NITROGEN BY THE FOLIN METHOD*

By Dale P. Osborn, B.S., M.D., Detroit, Mich.

WHEN several blood nitrogens have to be run at once, considerable time is wasted holding each pyrex tube over a flame waiting for the evaporation and digestion of the mixture to take place. For this reason an apparatus was constructed which has given us considerable satisfaction for over a year.

This apparatus, as can be seen in the illustration, consists of a rectangular box lying on its side, 14½ inches long, 8 inches high, and 4½ inches deep.

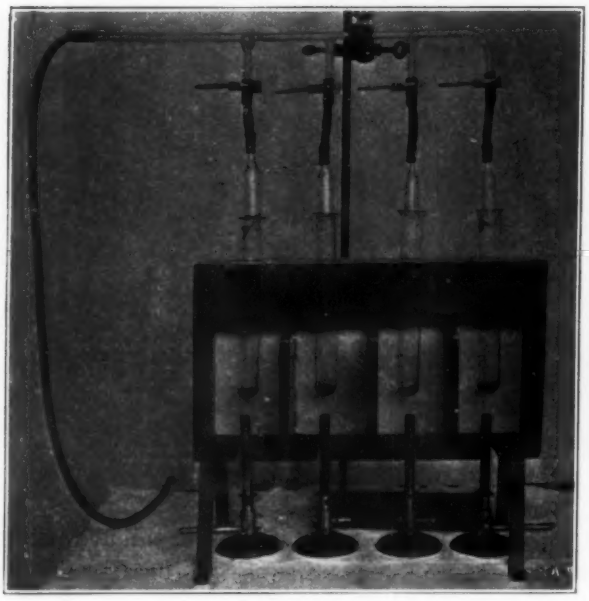

Fig. 1.—Front view.

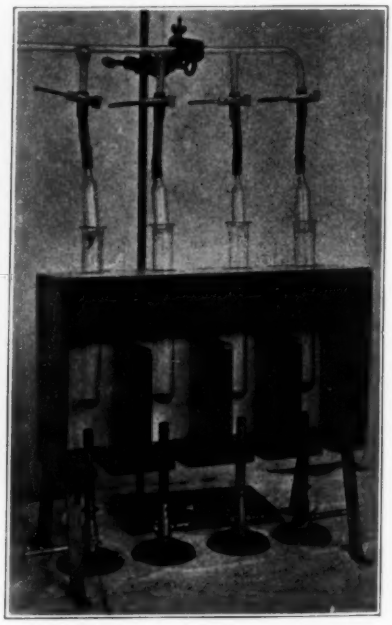

Fig. 2.—Side view, showing compartments.

It is divided into four compartments by stalls 4¾ inches high and ⅜ inches thick. These compartments will prevent annoying drafts.

The box is supported on four iron legs 4¾ inches from the table. A slot is made in the floor of each compartment ⅝ inch wide and 3½ inches deep, for the purpose of sliding in a Bunsen Burner. Four holes are drilled on the top of the box, one over each compartment. These holes are 1⅛ inches in diameter and 1⅛ inches from the back of the box. Four spring

*From the Buhl Memorial Laboratory, Harper Hospital, Detroit, Mich.
Received for publication, March 12, 1924.

tube holders are fastened inside the box on a strip of wood ¾ inch thick, 2⅛ inches from the top. The holders were taken from an old pipette rack.

The illustration shows how the suction apparatus is arranged, each inlet is attached by a rubber tube to a small funnel made by drawing out an ordinary test tube and breaking at the narrowest point. The mouth of the funnel tube will comfortably fit into a pyrex tube allowing just enough space for necessary draft. Suction is secured by an ordinary suction nozzle connected with a water tap.

The inside of the apparatus is painted black with the exception of the back, which is enameled white to give a good background for reading the end point of digestion.

Since using this apparatus, we find that we can make our nitrogen determinations in from one-third to one-quarter of the time formerly used. If one uses glass beads, the whole process may be carried out with the apparatus. However, since we do not use the beads, we evaporate the solution by hand over a Bunsen flame to the point of turning brown. The pyrex tube is then transferred to the box over a Bunsen microburner and the digestion is completed and the mixture cooled while other evaporations are done by hand and transferred to the box.

As soon as the brown solution turns green and clears, the microburner is quickly pulled out from under the pyrex tube along the slot in the bottom of the box. By the time each compartment has been filled, the first tube has cooled and may be taken out. The process is then repeated until all the solutions are digested. Water and Nesslers' is then added and the readings and determinations made.

A NOTE UPON A SIMPLE METHOD FOR THE REMOVAL OF NATURAL ANTISHEEP AMBOCEPTOR FROM HUMAN SERA*

By Robert A. Kilduffe, B.A., M.A., M.D., Los Angeles, Calif.

THE possible influence of the natural antisheep hemolysins present in human sera has been the subject of much investigation and discussion in the past and will probably continue to be discussed in the future, for, while the advisability of their removal when sera are tested for complement-fixation in syphilis in a single dose of 0.1 or 0.2 c.c. may be conceded, their exact status in quantitative tests utilizing graded doses has yet to be ascertained.

Kolmer,[1] for example, does not find their presence of practical importance in the quantitative technic described by him and states their influence as perceptibly affecting the results with his method to be "practically negligible." Detweiler[2] holds similar views with regard to methods using graded serum doses (0.2 c.c., 0.1 c.c. and 0.05 c.c.) and quotes Neill[3] among others in support of the view that their influence may be disregarded in methods *testing less than 0.1 c.c.*

*Received for publication, February 22, 1924.

Regardless of this phase of the discussion, which is not yet definitely settled, a large bulk of the literature has been concerned with the feasibility and practicability of methods proposed for the removal of natural hemolysins by absorption, especially as applied to relatively large numbers of sera.

Many have been proposed and objected to on either one or other of two counts: (a) The time consumed; (b) The development of anticomplementary properties in the sera after absorption.

Both of these objections are well founded and may assume serious proportions and the purpose of this note is to report experience with a method proposed by Kahn[4] and which has been found to be simple, rapid, efficacious, and without apparent influence in causing the development of anticomplementary properties.

The method consists in the addition of 0.05 c.c. (one drop) of washed and closely packed sheep cells to 1 c.c. of inactivated serum, the mixture being allowed to stand for ten minutes at room temperature when the serum is removed by centrifugation. The quantities of serum and sheep cells are approximated and need not be accurately measured.

This method has been used in a large number of sera in the course of an investigation of the influence of natural amboceptor upon Kolmer's quantitative method of complement fixation in syphilis and has been found entirely satisfactory for the purpose.

In each instance the sera were tested after absorption for the removal of natural amboceptor and in each instance this was found to be complete regardless of the amount present. The development of anticomplementary properties has not yet been noted and must be quite rare.

Kahn's method for the removal of natural hemolysins, therefore, is quite suitable for even large numbers of sera and does not appreciably add to the time required for the performance of the test.

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A BACTERIOLOGICAL INCUBATOR ROOM*

BY BENJAMIN WHITE AND ROBERT N. NYE, BOSTON, MASS.

EVERY laboratory in which large numbers of bacterial cultures are carried requires an incubating space greater than that provided by the usual type of incubators found in the market. From the standpoint of convenience and expense it is, therefore, preferable to devote a room to this

*From the Antitoxin and Vaccine Laboratory, Department of Public Health, Commonwealth of Massachusetts, Boston.
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purpose. The proper construction, insulation and heating of such a room present difficulties which have been overcome in a variety of ways, but many installations have called for more or less elaborate construction, with complicated heating and heating control devices, and their complicated nature frequently results in inefficient or expensive operation.

An incubating room built in this laboratory embodies certain features which have made for great simplification and which have proved to be highly efficient and economical. It is in the hope that some of the features of this incubator room may be helpful to others that this description is published.

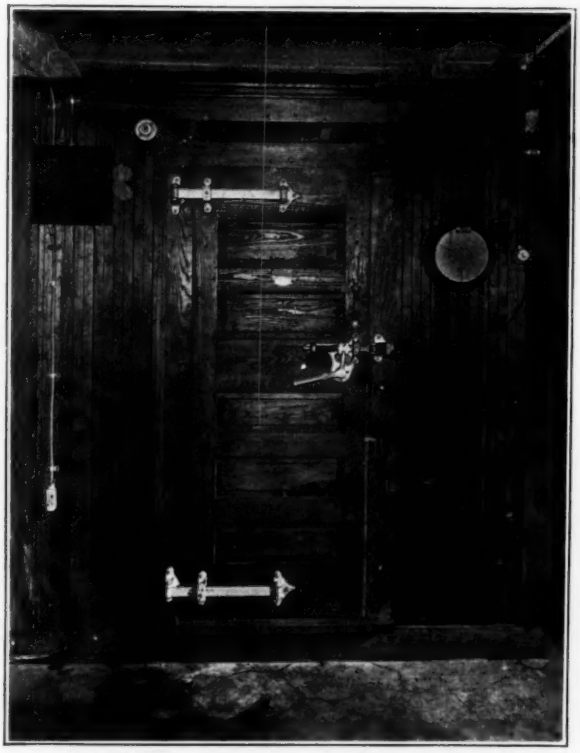

Plate 1.

1. *Construction:* The room is 9 feet long by 7 feet 8 inches wide and 8 feet high (outside dimensions). Walls, ceiling and floor are constructed of 7/8 inch matched boarding, followed inside by 2 inches of asbestos fibre loose filling, followed by a 2 inch thickness of asbestocel sheets, and the main inner surface is of 1/4th inch Transite asbestos wood. All joints are pointed with retort cement and the main surfaces are given a coat of filler, two coasts of flat white and a coat of white enamel. The walls, therefore, are fireproof and the inside surface is wholly waterproof, making thorough cleansing possible. This gives an interior space 8 feet long by 7 wide by 8 high, or 448 cubic feet, with walls capable of retaining the generated heat in the room with a minimum of loss, and also of protecting the chamber against external variations in temperature. (See plate 1.)

The room is provided with four tiers of slatted movable shelves of heavy galvanized iron, extending along the three walls of the room. There are in all twelve shelf sections, each 5 feet 8 inches long by 1 foot 10 inches wide, giving approximately 125 square feet of shelf surface. (See plate 2.)

In one end there is a door opening six by three feet into which a regular refrigerator door tightly closes.

The current consumption varies from 200 to 300 kilowat hours per month depending upon the season of the year.

2. *Heating Device and Temperature Control:* The heaters are divided into two sets, one on the left wall and one on the right wall of the incubator

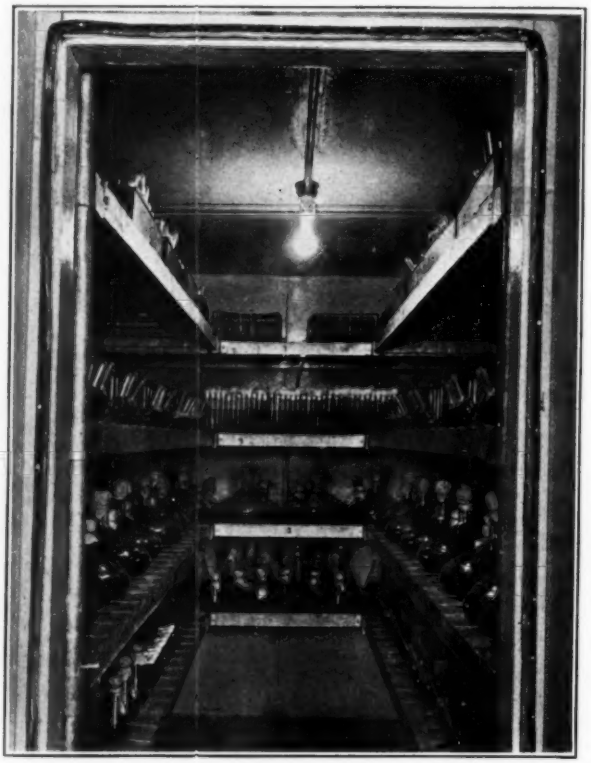

Plate 2.

room. Each set consists of constant heating units and an intermittent heating unit. These units are attached to and spaced from the walls by porcelain insulators. The constant heating units are placed about eighteen inches above the floor and the intermittent units six inches higher. In order to facilitate proper circulation of the heat there should be either a solid partition or baffle plates extending the full length of and about six inches from the side walls to within one foot of the floor and ceiling. The partitions or baffles, as well as the walls themselves, unless they are constructed of fireproof material, should be covered with sheet asbestos in the neighborhood of the heating units. In our particular instance ordinary refrigerating room baffles have been used. The frames containing the baffle plates are upside

down, so that the plates are reversed in direction and as the heated air rises from the units it has a tendency to draw in air from different levels of the room. With this arrangement there is a difference of approximately 2.0°C. between the upper and lower shelves. If an absolutely uniform temperature

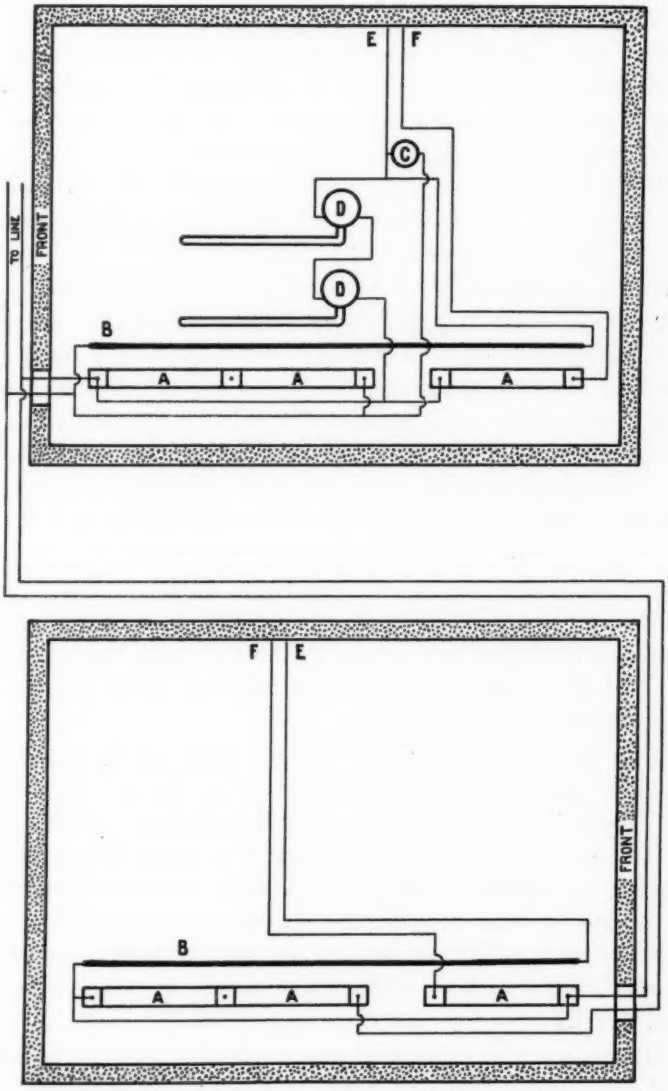

Plate 3.

throughout the room is required it can probably only be maintained by means of some sort of a forced draught.

The wiring (see diagram) is such that the space heaters, "A," are "on" all the time and they should be of such wattage as to maintain in the room a temperature of about 32°C. The proper wattage must be determined for each different installation. In the room described it was found that six

No. 3008 Cutler-Hammer space heaters—three sets of two in series (210 watts at 115 volts)—gave the desired amount of heat.

The intermittent heating units, "B," are made from bare No. 25 nichrome wire and extend the entire length of each wall. The coils should be of such length that their combined wattage is 50 to 75 per cent greater than that of the constant heaters. For our particular room a total wattage of about 350 with the coils connected in multiple has proved satisfactory. It is immaterial whether such a value is obtained by connecting two 700 watt coils in series or two 175 watt coils in multiple. In wiring, one side of the line is brought direct to the thermoregulators and is then carried to the heating coils. A lamp socket, "C," is connected to the line after it leaves the controls, and, with a bulb screwed in, serves as an indicator of whether or not the coils are "on."

The thermoregulators,[1] "D," are of the make and break type and are connected in series. One is adjusted to operate at 37.5°C. and the other at 38.0°C. Ordinarily the latter remains "on" all of the time; but, if the former ceases to function properly, the latter commences to operate as soon as the temperature of the room reaches 38.0°C. These thermoregulators handle all the current (350 watts) required to operate the intermittent heating units. An *alternating current must be used* because on direct current the thermoregulators will arc if the current consumption is greater than 75 watts.

All the wires leave the incubator room through two openings. The wires from the two openings are led to a fuse box on the outside of the front wall of the room and are connected to the main line. Only two wires, "E" and "F," are required to connect the two sides inside the box and these are carried over on the ceiling.

Ventilation is accomplished by two openings about six inches square, one at the floor level on one side and another at the ceiling level on the opposite side. Each is supplied with a sliding shutter. If the determination of the constant wattage necessary to maintain a temperature of 32°C. is carried out during the winter months, the shutters should be practically closed. Otherwise, during the summer months, owing to decreased radiation and increase in temperature of the incoming air, this wattage may be sufficient to heat the room above 37.5°C. and this increase in temperature cannot be compensated for by opening the shutters wider.

A continuous graphic record of the incubator room temperature is kept by means of a recording thermometer[1]. A fresh chart is put on every twenty-four hours and the completed charts are kept on file.

The incubator room temperature at any one level is constant within a small fraction of one degree Centigrade. Some idea of this constancy may be obtained from the following data. During the winter months the "on"

[1]Manufactured by the Cobb Electric Appliance Co., Forest Hills, Mass. The action of the thermoregulators is dependent upon the expansion of acetone. They should be adjusted by the manufacturer so that at 37.5°C. the pressure is not less than 100 lbs. nor greater than 200 lbs. per square inch. They will satisfactorily handle 500 watts (A.C.) and will probably handle as high as 1000 watts (A.C.).

[1]Manufactured by the Foxboro Co., Foxboro, Mass.

and "off" periods of the intermittent heating units are on the average two minutes and one minute fifty-five seconds respectively with the bottom ventilating opening closed and with the top open about two centimeters. Furthermore, with both the constant and intermittent heating units "on" practically eighty minutes are required to raise the incubator room temperature from 36.0° to 37.0°C. Since the "on" period of the intermittent units represents the time required to raise from the minimum to the maximum, as determined by the thermoregulator, then the actual rise in temperature must equal approximately $\frac{2}{80}$ of 1.0° C. or 0.025°C. This means that the actual temperature at one particular level is constant within the almost unbelievable figure of $\pm$ 0.013°C.

This system of constant and intermittent heating has been developed in an effort to eliminate the use of relays. For over a year attempts were made to heat the incubator room with bare wire intermittent heaters. This required a wattage of about 1600 and necessitated the use of a relay. During this time four different kinds of relays were tried out and every one eventually failed to operate. The present system has been in operation a year and has not given the slightest trouble.

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EDITORIALS

The Kottman Reaction for Thyroid Activity

THERE has never been a time in the history of medicine when the avenues of approach in the diagnostic study of a particular case have been so numerous, so varied, and, withal, so informative when properly applied and keenly interpreted.

There has never been a time, likewise, when the tendency to rely more or less entirely upon laboratory findings has been more marked in the rank and file of the profession—a tendency to be decried and discouraged.

The value and usefulness of any method of laboratory examination is in direct proportion and indissolubly related to the care with which it is applied and the acumen with which the findings are interpreted and correlated with all the other data in the particular case. Particularly is this true in those conditions whose mechanism and modus operandi are not yet an open book, an excellent example of such conditions being the symptom complexes associated with thyroid dysfunction, as an indication of which the Kottman reaction has recently been the subject of some study.

As a result of work done on the physicochemical changes of the blood in pregnancy, Kottman advanced the theory that physicochemical changes must exist in the serum in thyroid dysfunction and that the differences would be in the nature of changes in the state of dispersion of the serum colloids.

Dispersion is the incorporation of particles of one substance into the body of another. If the particles are fine a solution occurs; if somewhat coarser, a gelatin results; and if still more coarse, an emulsion is produced.

Kottman assumed that the size of the colloid particles of the blood and body fluids is influenced by the amount and activity of the thyroid secretion, being small in the presence of an excess of secretion, because of the presence of a protective element, and larger in the presence of hyposecretion because of the lack of the protective element.

For the demonstration of the assumption he took advantage of certain elementary principles of photography.

In the making of a photographic film gelatin is mixed with silver iodide or bromide. When freshly mixed the substances are in a highly dispersed state and, therefore, not very photosensitive; during the period of "ripening" the colloidal particles of silver salt become larger and more sensitive to light, and it is upon this principle that the Kottman test depends, the gelatin being replaced by the serum to be tested. The size of the colloidal particles of silver iodide is estimated by the sensitivity of the mixture to light, the particles becoming smaller or larger in accordance with the amount of protective element present in the serum. The greater the photosensitivity, the poorer the protective property of the serum, presumably because of the coarser colloidal dispersion of the serum, and *vice versa.*

The technic of the test is simple: To one cubic centimeter of clear, freshly collected serum are added 0.25 c.c. of a 0.5 per cent solution of potassium iodide and 0.3 centimeter of a 0.5 per cent solution of silver nitrate.

The resulting suspension of silver iodide in the serum is now exposed for five minutes to a 500 Watt Mazda lamp at a distance of 25 cm. (any light of equal intensity may be used), when 0.5 c.c. of a 0.25 per cent solution of hydroquinon is added and the color reactions observed at five minute intervals. Diffused daylight does not interfere with the reaction and a dark room is only theoretically necessary.

The test tube should be carefully cleaned and of uniform size, 1.5 by 8 cm. being convenient, as allowing of gentle agitation.

The reagents must be freshly prepared and accurately measured as an excess of either the iodide or the silver nitrate affects the time of appearance of the reaction and its degree.

In normal serums the silver iodide is reduced to free silver promptly with the production of a brown color; in hyperthyroid cases this reduction is retarded, the serum remaining yellowish; in hypothyroid cases the reduction is accelerated.

The administration of bromides prior to the test interferes with the reaction and must be guarded against.

The test has been studied in a fairly large number of thyroid cases with relatively constant results.

Petersen, H'Doubler, Levinson, and Laibe[1] found the reaction delayed in 65 of 70 hyperthyroid cases, the controls showing a very close indication of thyroid activity. In two cases the reaction was only slightly retarded; in two cases it was normal, and in one case accelerated. All of the last three cases were active with typical symptomatology and clinical findings including a high BMR.

The same observers[2] in an experimental study of the conditions affecting the occurrence of the reaction, found that the administration of iodides affected the test though not so markedly as bromides; that fright caused marked retardation, while severe trauma without fright was without effect; that thyroidectomy increased the reaction; while irradiation completely retarded it without concomitant evidence of hyperthyroidism. After the injection of thyroxin there was retardation which, however, was not apparent until after a latent period of several days. In dogs thyroidectomy was without effect during a period of 19 days; irradiation showed an early increase in the reaction with a later decrease not, however, comparable to that seen in true hyperthyroidism; with chloroform necrosis of the liver the reaction was retarded giving rise to the assumption that either extensive tissue injury may bring about serum alterations similar to those occurring in hyperthyroidism, or that there may be a mobilization in the blood stream of substances stimulating thyroid activity. Bacterial intoxication by the injection of colon bacilli or intoxication following the injection of protein caused retardation but the mere ingestion of peptone caused no change. Following hemorrhage and excessive exercise there was also a change in the direction of retardation, probably due to alterations in the globulin ratio and alterations in the pH balance. These investigators advance the assumption that thyroid dysfunction alters the cellular metabolism which in turn alters the physical properties of the serum, or else that in hyperthyroidism the serum is more protective than normally for reasons yet to be adduced.

Saunders[3] in a series of 400 cases among the insane found 105 giving retardation, approximately fifty per cent of which were frank cases of hyperthyroidism; the remainder had very indefinite symptoms, sometimes merely a somewhat rapid pulse. In this group the feeding of thyroid and the administration of adrenalin brought out a few more cases of probable hyperthyroid activity.

The test is thus seen to have a decided value and warrants further and extensive study. In the final evaluation of any method, however, of primary importance is the determination of the results obtained in an extensive series of conditions other than that for which the test was devised and, also, of the possible factors influencing and affecting its delicacy and reliability.

Of great importance, therefore, is the paper by Morse and Fitch[4] who call attention to a very important factor affecting the results and the reliability of the Kottman reaction.

Having corroborated the observations of others as to variations in the

reaction of serums when kept, which caused a tendency toward acceleration of the color changes, and also noting that the color change was first noted on top of the serum and not uniformly throughout the tube, the thought of carbon dioxide as possibly influencing the reaction occurred and was subjected to experimental investigation.

Carbon dioxide affects the acidity of the serum thus influencing the balance of chemical reaction which is at the bottom of photochemical sensitivity.

These investigators, therefore, tested the effect of aeration of the specimens demonstrating, as a result, an acceleration of the reaction in most cases, though in some a retardation occurred, thus suggesting that changes in a still more fundamental variable—possibly the pH concentration—might be concerned.

Their observations explain in some degree the occurrence of anomalous reactions and warrant their contention that, not only must the reliability and delicacy of this test be restudied in the light of these factors, but also that in the future samples for this reaction must either be collected under oil or with the precautions customary in estimations of the carbon-dioxide content of the plasma, or that the test must be so modified as to eliminate this error.

The test is certainly not without value but its final evaluation will depend upon an extensive reinvestigation of the entire subject.

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—*R. A. K.*

An Increase in Size of Journal

IT is necessary to increase the size of the Journal in order that the many excellent papers received be published within a reasonable length of time.

To accomplish this between sixteen and thirty-two pages will be added to each issue, thus increasing by two hundred pages the amount of reading matter that will be given to our readers during the year. This increase necessitates our raising the price of the Journal beginning with the October issue one dollar per year. All subscribers will note this on our renewal statements.

BOOK REVIEWS

(Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.)

*Intravenous Therapy**

THE first two hundred pages of this book are devoted to a brief historical résumé of the administration of saline solutions, the various methods of direct and indirect transfusion in adults and in infants, and the intravenous administration of arsphenamine and related compounds. These subjects are ably discussed with emphasis placed upon the comparative advantages of the different methods, the drawbacks, dangers, reactions, etc.

In the remaining three hundred pages we find an alphabetic classification of all sorts of diseases in which intravenous therapy of any nature whatsoever has been tried. As a rule the etiology, pathology, symptoms, diagnosis and treatment of these various diseases are enumerated in a superficial way. Discussion of treatment is not limited to intravenous treatment but includes other recognized methods. In the vast majority the intravenous portion of the treatment is of least importance and often its value has not been demonstrated.

We trust that the future will not bring us specialists in intravenous therapy.

Infection, Immunity and Biologic Therapy†

THE third edition of Kolmer's work, covering the principles and the practice of the recognized serologic and immunologic laboratory procedures that have developed up to the present time has grown to a volume of over twelve hundred pages. Such thoroughly recent developments as the flocculation or precipitation reactions for syphilis, and the Kolmer quantitative Wassermann technic receive full consideration.

Kolmer finds that the precipitation reactions are not as sensitive as the complement-fixation reactions when conducted with technically correct and acceptable technic; that they are more subject to misinterpretation and should not be relied upon alone for the serum diagnosis of syphilis. He suggests that they may be useful as controls, and recommends as the more reliable, the third modification of the Meinicke, and particularly the Sachs-Georgi and the Kahn reactions.

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*Intravenous Therapy. By Walton Forest Dutton, M.D. Medical Director, Polyclinic and Medico-Chirurgical Hospitals. Graduate School of Medicine, University of Pennsylvania. Cloth. Price $5.50. Pp. 542. Illustrated with 59 half-tones and line engravings, some in colors. Philadelphia. F. A. Davis Co., Publishers, 1924.

†Infection, Immunity and Biologic Therapy: By. John A. Kolmer, M.D., Dr. P. H., D.Sc. (Hon.) Professor of Pathology and Bacteriology in the Graduate School of Medicine, University of Pennsylvania; and Member of the Research Institute of Cutaneous Medicine, Philadelphia. With Introduction by Allen J. Smith, M.D., Sc.D., LL.D. Professor of Pathology in the School of Medicine of the University of Pennsylvania. Third edition. Pp. 1210; 202 original illustrations, 51 in colors. Drawn by Erwin F. Faber, Instructor of Medical Drawing, University of Pennsylvania. Cloth, Price $12.00. W. B. Saunders Co., 1924.

The section devoted to allergens and allergic disease is usually complete and should be of interest not only to laboratory workers but also to clinicians who will find therein a clinical discussion of allergic diseases and of their practical treatment. Various forms of vaccination and nonspecific protein therapy are described in detail.

Contrary to the prevailing conception, this work should be of nearly as great value to the clinician as to the laboratory worker because of its wealth of information on the diagnosis and treatment of a great variety of diseases.

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*Clinical Laboratory Diagnosis**

THIS volume, first presented by its author to the profession in 1913, has developed with the evolution of laboratory methods and in its latest edition incorporates those newer methods which have to a certain extent revolutionized our clinical laboratory procedure. The treatment of the subject follows rather closely that found satisfactory in earlier editions and the new work has been assimilated therein. A supplement is devoted to blood chemistry methods. Many of the tabulations should be very convenient for handy reference.

This work should, like its predecessor, find its way to a large number of laboratory shelves.

Fighting Foes too Small to See†

ALTHOUGH the title appears rather heavy and might perhaps frighten away prospective readers, between the covers we find a thoroughly interesting popular exposition of the warfare against infectious disease. The illustrations are particularly good. The author flings an occasional dart at his fellow-doctors, such as a statement that many of the most important discoveries were made by individuals not physicians and that doctors were sometimes the last to understand them or to profit therefrom. While this is perhaps not entirely undeserved, when we consider that the laity has as a whole been still less willing to accept the newer doctrines of epidemiology and parasitology, and since the author is addressing a lay audience, we are inclined to a slight resentment. This, however, is a minor consideration and we trust that the book will receive the large audience that it deserves.

The Constitutional Factors in Dementia Precox‡

DISEASE in general has been classified as organic or somatic, and psychic. The latter is considered as due to inherent functional perversions of the psyche rather than to organic disease of the central nervous system. However,

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*Clinical Laboratory Diagnosis: By Roger Sylvester Morris, A.B., M.D., Professor of Medicine in the University of Cincinnati and Director of the Medical Clinic of the Cincinnati General Hospital; formerly Associate Professor of Medicine in Washington University, St. Louis; Associate in Medicine, The Johns Hopkins University and Assistant resident physician Johns Hopkins Hospital; Instructor in Medicine, University of Michigan. Cloth. Pp. 456. D. Appleton & Co., New York, 1923.

†Fighting Foes Too Small to See: By Joseph McFarland, M.D., Sc.D., Professor of Pathology in the Medical Department of the University of Pennsylvania. Cloth. Pp. 309. Illustrated with 64 engravings. Price $2.50. F. A. Davis Co., Publishers, Philadelphia, 1924.

‡The Constitutional Factors in Dementia Precox. By Nolan D. C. Lewis, M.D. Nervous and Mental Monograph Series No. 35. Paper. Pp. 135. Nervous and Mental Disease Publishing Co., New York and Washington, 1923.

one psychiatric disturbance after another has been found to be associated with, if not actually due to, an organic disturbance of the central nervous system. Examples are to be found in general paresis, epilepsy, infection and exhaustion psychoses, etc. How many more so-called pure affections of the mind will eventually be shown to be associated with somatic disease? Dr. Lewis has studied dementia precox with this question in mind and has found that in the greater number of his cases there is an associated disease of other organs of such a nature as to be intimately associated etiologically with the mental abnormality. The most frequent concomitant disease appears to be tuberculosis or the history of this disease in the patient's antecedents. But the conditions which Lewis points to as being particularly characteristic in dementia precox are an hypoplasia of the circulatory apparatus with usually a status lymphaticus constitution and anatomic deficiency of the thyroid and adrenals, less constantly of the gonads and pituitary. The circulatory hypoplasia appears to be due to an arrest of development around the age of puberty quite analagous to the retardation of mental development at the same time. On technical grounds one might take exception to the author's use of the word "aplasia" in speaking of underdevelopment.